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ZINC AS A NUTRIENT FOR PLANTS[1]

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(WITH FIVE FIGURES)

As early as 1869, RAULIN (**43, 44**) presented strong evidence that zinc is essential in the culture medium for at least some fungi. He thought it was not merely a stimulant but an essential element, and that growth when zinc is not added to the medium is due to the presence of zinc as an impurity. JAVILLIER (**22, 24, 25**) emphasized this point of view and showed that, besides being an impurity in the purest chemicals that could be purchased, zinc was apt to be present in glassware. When he grew *Aspergillus* in Bohemian glass or quartz without addition of zinc to the medium, there was much less growth than when he grew it in Jena glass. Other workers (**14, 30–34**) did not find results in harmony with the views of JAVILLIER, probably because they did not exclude as many of the sources of zinc as an impurity. This view seems justified, especially by the work of STEINBERG (**50–57**), in which he was able to reduce the amount of zinc as an impurity so much that growth by *Aspergillus* when zinc was not supplied was less than 10 per cent of that when it was supplied. Some workers before STEINBERG thought of zinc not as an essential element but merely as a stimulant for fungi: zinc present as impurities was enough for apparently normal growth, so that the additional growth when zinc was added seemed merely a stimulation (**8**).

[1] A summary of work that has been or is to be published in cooperation with D. R. HOAGLAND, P. L. HIBBARD, J. C. MARTIN, and P. R. STOUT. Paper presented at a Symposium on Mineral Nutrition, December 1936 Meetings of the A.A.A.S.

RAULIN (44, 45) thought zinc was an essential element for higher plants also. JAVILLIER reported work with water cultures in which minute quantities of zinc in the culture medium caused increased growth. Other workers before and after this obtained suggestions of response to zinc by higher plants in water culture and in sand culture. The careful work of MAZE (35–37) with maize plants grown in water culture, and the work of SOMMER and LIPMAN (47, 48, 49), in which great care was used to prevent the plants from obtaining a zinc supply from the glassware, the water, the dust of the greenhouse, or the chemicals in the nutrient solution, seem to establish beyond question the requirement of zinc as a nutrient by some plants if not by all. HAAS (18) reported a benefit from zinc to lemon trees grown in water cultures. More recently HOAGLAND *et al.* (20, 21) have described, from water cultures, zinc deficiency symptoms on several other plants, including young apricot trees.

There have been reports of increased growth and yield by plants in the field in response to applications of zinc to the soil, and reports of disease symptoms cured by such applications. JAVILLIER (23) apparently found 5.5 to 55 pounds of zinc sulphate per acre to increase the growth of wheat, oats, maize, lupines, and peas. Beginning in 1927, workers in Florida (5, 6, 7) reported benefits to maize, peanuts, peas, millet, and some other annual plants, from treatment with zinc. Zinc deficient maize plants showed chlorotic streaks between the veins; this disease was called white-bud.

MOWRY and CAMP (39) found that zinc sulphate, as little as 0.5 pound to the tree, applied to the soil, would cure serious injury to tung oil trees, and even 0.25 pound each caused striking improvement to mottle-leafed Satsuma orange trees in Florida.

ALBEN *et al.* (2, 3) in Louisiana, DEMAREE *et al.* (15) in Georgia, and FINCH and KINNISON (16) in Arizona have found that zinc sulphate will cure rosette of pecan trees, a disease that causes, in addition to rosettes of small leaves with shortened internodes in spring, yellow mottling between the leaf veins, later some crinkling of leaves, and sometimes dying back of the shoots.

CURING AN ORCHARD DISEASE WITH ZINC.—In 1931 and 1932, four different groups of workers discovered independently that treatment with zinc, through the soil and in other ways, will cure a serious

orchard disease, one that affects trees of some kinds in some areas and of all kinds in some areas from Florida westward through the Gulf states, Texas, Arizona, and California, and northward at least to the Canadian line. Many millions of dollars worth of land otherwise excellent for orchards are worthless unless zinc is supplied to prevent or cure this disease. On the pecan and the apple it is called rosette; on the citrus fruits, mottle-leaf; on the walnut, yellows; on grapevines, stone-fruit trees, and some other trees, little-leaf. Trees are cured, however, with zinc whose symptoms are not described by any one of these terms; and not all rosetting, mottling, or yellowing is cured with zinc. The only sure means of identifying the disease is the response of the trees. In this paper, therefore, I shall call the disease zinc deficiency, even though the evidence now available may not prove beyond question that it is due merely to lack of zinc as an essential element.

Observations

In California (10–13), following the work of HAAS and REED (19), among the first trials were treatments with a mixture of many minor elements including 90 pounds to the acre of zinc sulphate, a larger quantity than was necessary to cause healthy growth of injured plants in Florida soils. There was no evidence of response, however, and, as in Louisiana (2, 3), the effectiveness of zinc in curing this disease in California was discovered somewhat accidentally (10) by using ferrous sulphate that contained a considerable amount of zinc. Since this discovery in 1931, plants have been treated continuously in more than fifty districts over an area more than 400 miles long in the San Joaquin, Sacramento, and other valleys. Trees have been treated with zinc sulphate through the soil, through holes in the trunk, by driving pieces of zinc or galvanized iron into the trunk or branches, by spraying the foliage with zinc sulphate and lime or with zinc oxide, and by spraying when the foliage was off with zinc sulphate or zinc chloride solution.

Soil treatments tend to be more effective the nearer the zinc compound is placed to the trunk of the tree. In the soil with the lowest fixing power for zinc of any studied in this investigation, 1000 pounds of zinc sulphate to the acre spread over all the soil would cure nearly

all the trees and keep them healthy for about three years; 300 pounds to the acre all within 2 feet of the trunks was more uniformly effective and the benefit lasted as long; in some soils, 2500 pounds to the acre all within 2 feet of the trunk was not so uniformly effective and the benefit never lasted so long. Adding with the zinc sulphate one to three times as much ferrous sulphate, aluminum sulphate, or calcium sulphate, slightly reduced the amount necessary to cure a tree but did not prolong the time before symptoms would show again. Mixing ammonium sulphate with the zinc sulphate also increased its effectiveness slightly, but mixing complete fertilizer (**17**) with it seemed to reduce its effectiveness a little.

Zinc sulphate powder packed into holes around the trunk about 3 inches apart, about 2-3 gm. to the hole, and covered with a wax that is harmless to the tissue, has never failed to cure a tree that showed zinc deficiency. The benefit usually lasts at least three years, to trees in soil with a high or a low fixing power. A considerable amount of sapwood is killed in the trunk, however, and some of the holes may remain open and permit the entrance of heart rots.

Driving pieces of zinc or of galvanized iron or zinc coated nails deep enough into the trunk or branches to hold well, 15 to 20 pieces nearly an inch wide or 50 to 60 zinc coated nails for each inch of trunk diameter, will cure zinc deficient trees of all the kinds tested except citrus. On stone fruits and the walnut the pieces should be kept an inch apart or there may be killing of the bark and cambium between them. The benefit seems to last longer than that from any other method of treatment, although it has been tried for only four years. This seems to be the most satisfactory method of treating walnut trees and cane-pruned grapevines. Pieces driven into one branch will cure the parts of that branch above the pieces but not the parts below them.

Spraying the foliage with zinc sulphate, 10 pounds or somewhat less with half as much hydrated lime in 100 gallons of water, has been found surprisingly effective for citrus trees (**27–29, 40–42**). It is moderately effective for apricot trees and grapevines, but almost completely ineffective for other kinds of deciduous trees tested (**12, 13**). For citrus, zinc oxide seems a little more effective than

zinc sulphate and lime, and it can be combined with other sprays, including oil sprays.

Spraying in winter (13), after all leaves are off and before the buds begin to swell, with 25 to 50 pounds of zinc sulphate, considerably less of zinc chloride, in 100 gallons of water, has cured apple trees and stone-fruit trees but has failed to cure walnut trees (fig. 1).

FIG. 1.—Walnut tree showing zinc deficiency symptoms. There are no rosettes, but the leaves are rather small, mottled, and crinkled.

Zinc chloride may have benefited walnut trees somewhat. Spraying must be repeated each year, at least during the first four or five years after treatment of badly affected trees begins.

In soil treatment, fixing power for zinc determines the amount that must be applied for a cure. In treatments above ground, fixing power for zinc and degree of zinc deficiency have little to do with responses, except that in dormant spraying stronger solutions are necessary in the first and sometimes in the second year of spraying, if the shoots have been killed back badly by lack of zinc.

Following treatment during late summer, autumn, or winter, in the soil, in holes in the trunk, or by driving zinc into the trunk or branches, the rosettes of small leaves show in the first spring; improvement begins to show two or three months after growth starts. Following winter spraying, the leaves show healthy from the beginning of spring growth: buds that without the spraying would open into rosettes of small, mottled leaves expand after the spraying into shoots bearing normal leaves.

The best treatment for grapevines pruned to spurs with two or three buds seems to be to spray or swab them immediately after pruning with 2 pounds of zinc sulphate to a gallon of water; spraying unpruned vines or vines pruned late enough in winter to bleed, even with such strong solutions, is not effective. On trees, pruning wounds are not so helpful toward getting zinc into the wood.

Symptoms of zinc deficiency and susceptibility of different species

By use of all these methods, one or more of which have been successful on all species, it has been possible to determine with reasonable certainty what symptoms may disclose zinc deficiency for the different orchard species under different conditions (fig. 2). The most dependable symptoms on deciduous trees are the rosettes of small, stiff, nearly sessile leaves in the first flush of growth in spring. These leaves may or may not be mottled and they never become more than 1 inch long or about 0.25 inch wide. From below them, further below on apple trees than on stone-fruit trees, shoots tend to start that may bear healthy green leaves throughout the summer, the appearance of the trees in late summer often being better than that of healthy trees, because they have not been bedraggled with fruit. Trees affected more severely may bear leaves on these later shoots more or less mottled from the beginning, with badly mottled, small, and abnormally shaped leaves toward the end of the season's growth. These same symptoms were observed by Hoagland *et al.* (20) on apricot trees grown in zinc deficient water culture. Some trees begin to die back when they have been affected a year or two, others after many years. Spring rosettes of small leaves are rare on cherry trees and Persian walnut trees, and are not

always shown by zinc deficient citrus trees. In fact, old, unpruned trees of all kinds rarely show typical rosettes of small leaves in

FIG. 2.—Zinc deficient apple tree photographed in July, showing rosettes of small leaves and little other growth. A tree mildly affected would have a considerable number of normal shoots starting from below the rosettes.

spring except on suckers. In orchards of kinds such as the almond, that are pruned lightly so that the shoot growth is very short, it is

often impossible to distinguish the symptoms of zinc deficiency from those of other influences, such as nitrogen deficiency, crowngall, or nematodes, that cause leaves to be small.

If any fruit sets on badly affected branches, it is mostly worthless and shows symptoms characteristic of the species. Peaches and Japanese plums, for example, are smaller, more flattened, and more beaked than normal ones; apricots are small and less flattened (fig. 3); lemons are small, oblong, and pointed. There is also a tendency for stone fruits to have brown areas in the flesh and to break down early. Walnut trees bear small poorly filled nuts, with tough, somewhat pliable shells instead of the normal brittle ones.

There is always a reduction in the number of fruit buds on trees rather badly affected, but stone-fruit trees usually have enough flowers for a fair crop if a good percentage of them set fruit. Usually, however, nearly all the flowers fail to set on the badly affected parts of the tree. Often the upper part of the tree is badly affected and bears only a few abnormally shaped fruits, while the lower part bears normal foliage and at least a moderate crop of fruits nearly all of which are approximately normal in appearance. A winter treatment with zinc that causes the first foliage in spring to be normal also causes the flowers, if there are any, to set fruit and causes the fruit to be normal in size and form.

All orchard and ornamental trees and shrubs grown in the valleys of California seem to show injury if the zinc deficiency is great enough, but the differences in susceptibility are large. For example, peach trees may be dying from insufficient zinc in a soil on which Oriental plane trees and grapevines of some varieties are healthy, but the situation is so severe in some soils that plane trees and grapevines are seriously injured unless they are treated with zinc. The most susceptible seems to be the sweet cherry tree, which may be dying badly from insufficient zinc in soils on which trees of all other kinds are healthy. Then following in order seem to be the apple, the Japanese plum, possibly the Domestica plum, the peach, the walnut, the apricot, the avocado, citrus fruits, some varieties of grapes, and the fig. This may not be the true order in all cases. I cannot place, from experience, the pear or the pecan, but from limited observation they seem more susceptible than the walnut.

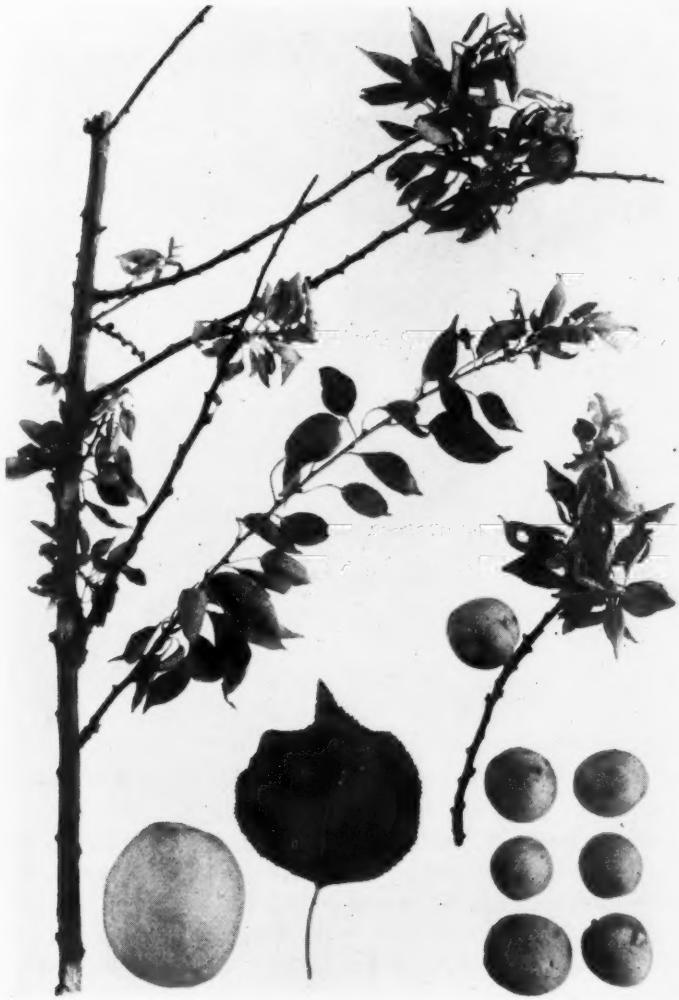

FIG. 3.—Abnormally small leaves and fruit from zinc deficient apricot tree. The fruit and leaf at lower left are from a tree which does not show symptoms of zinc deficiency.

ZINC DEFICIENCY IN CORRAL AREAS

In California, soils in which trees are most apt to show zinc deficiency are usually sandy or gravelly. Trees on compact clayey soils rarely show it except where there have been old corrals or where there has been heavy manuring for hotbeds or in truck growing, and where there have been Indian camps. In such areas trees tend to show rosettes of small leaves and other symptoms of zinc deficiency younger than even in the sandy soils. A few groups of trees on such areas have been completely cured with zinc, but they had been sprayed with Bordeaux mixture. Trees in other such areas have made striking response to zinc but have shown other symptoms than those of zinc deficiency. Copper and manganese in addition to zinc are being tried this year.

Four annual applications of 60 tons of fresh horse and cow stable manure to the acre or the equivalent amount of potassium, of phosphorus, or of nitrogen, have not yet produced symptoms of zinc deficiency on healthy trees or grapevines in soils that require old corral or hotbed influences to induce these symptoms. Perhaps time is required for such influences to develop in the soil. The element found in greatest excess in corral soils, and apparently in Indian camp soils, is potassium. Bringing the content of potassium to well above that of corral or Indian camp soils by application of 4 tons to the acre of potassium carbonate has not injured the trees in the least.

WHY SOILS SOMETIMES FAIL TO SUPPLY ZINC ENOUGH FOR TREES

In the soil studied most, peach trees made exceptional growth during the first five years and bore 14 tons of fruit to the acre in their fifth summer. In that year zinc deficiency symptoms began to show in the tops in late summer. In three more years many of the trees were dying. New trees planted in the position from which these were pulled would show zinc deficiency symptoms in their first summer in the orchard and never made much growth unless they were treated with zinc. Analyses by HIBBARD indicate that there are more than 3000 pounds of zinc to the acre in this soil within the root zone, and that at seven years of age the orchard had removed little if any more than a half pound to the acre in roots, top, and

fruit. At first these trees had obtained zinc enough for excellent growth and had removed only an insignificant part of the zinc supply in the soil. Why could they not obtain enough zinc after four years and why could not young trees following them obtain enough? Manure in a corral must add some zinc to the soil. Why does it reduce the supply obtained by the tree? Alfalfa growing among the trees will prevent zinc deficiency symptoms (**38**) in many western orchards and reduce the severity in all. It seems to increase the amount of zinc taken in by the tree. How does alfalfa exert this influence?

ALBEN and BOGGS (1), working in Texas and Louisiana, seemed to find that rosette, when found on pecan trees growing in an acid soil, could be explained by a very low total zinc content, about 116 to 136 pounds to the acre in the root zone, and that when found on trees in a basic soil with a much larger quantity of zinc it could be explained by the insolubility of zinc at such reactions, pH 7.4 to pH 8.5.

In California, trees on rather clayey soils with a reaction of pH 8.0 to pH 9.0 rarely show zinc deficiency, except on corral areas; most of the moderately basic soils on which trees do show zinc deficiency symptoms have high fixing power for zinc added to them in soluble form in the laboratory, but so also have some neutral and acid soils. In a district where the soil has the lowest fixing power for zinc of any so far studied, the reaction is usually pH 7.0 to pH 7.3, and nearly all stone-fruit trees show pronounced deficiency symptoms and will die young if zinc is not applied to them. Bringing the reaction down to pH 6.2 at the surface and pH 6.5 at a depth of 4 feet by application of sulphuric acid did not improve zinc deficient trees growing in this soil. One soil was found to have a very high fixing power for zinc and has an acid reaction, pH 5.9 to pH 6.4. In this soil 1500 pounds of zinc sulphate to the acre spread on the surface was rendered unavailable so quickly that apple trees were never cured completely by it, although less than 75 gm. of zinc sulphate in holes in the trunks cured large trees in the same orchard and has kept them healthy during more than four years, without retreatment. It may be that much of the zinc in this soil and of that applied as zinc sulphate is fixed only in replaceable combinations, as a considerable amount of the zinc applied by JONES, GALL, and

BARNETTE (**26**) to acid soils was, and that something else prevented the trees from taking it out of this fairly easily available combination fast enough for their needs. In fact it seems probable that in all these sandy or sandy loam soils, as well as in corral areas, in the Pacific slope where zinc deficiency causes so much loss, the explanation must be sought in some influence outside of purely inorganic chemistry. If the reaction of the soil and its total zinc content determined the supply to the growing points of the plant, then it would be expected that annual or herbaceous plants would show zinc deficiency where trees do. Yet many of the soils on which trees die young unless they are treated with zinc are exceptionally good for annual plants and alfalfa, without the application of any zinc (fig. 4).

In HOAGLAND'S (**20, 21**) studies, in the cool summers of Berkeley with their mild sunlight, neither fruit trees nor annual plants have tended to show zinc deficiency symptoms when grown in soil taken from near the surface of any of the areas where the disease is severe in the orchard. However, when the soil was taken from 3 to 5 feet deep in some of the areas and the plants were grown during the summer months in a greenhouse, or outside but protected from cool ocean winds, young apricot trees and many annual plants, including sunflowers, showed zinc deficiency symptoms. Maize showed typical white-bud. The symptoms were corrected by addition of zinc to the soil. Out of doors in the cool ocean wind the symptoms have not yet developed, and they develop slowly, if at all, during the winter months in the greenhouse. It seems that temperature and the amount of daylight may have something to do with development of these symptoms.

ROOT INJURY IN ZINC DEFICIENT ORCHARDS.—Many facts suggest that something in these soils prevents the roots either from absorbing available zinc or from transporting it to the tops. In these injured orchards there is some dying of the roots on trees that have been badly affected for a number of years, so badly affected that weak foliage might explain the root injury. However, trees affected only badly enough to prevent fruiting, not badly enough to prevent them from holding a considerable leaf surface throughout the summer, showed no injury to the roots that could be detected by field examination in deep trenches. Potassium seems to be leached out of the

bark of the root of zinc deficient trees until it contains only 10 to 25 per cent as much potassium as the bark above ground or as the bark on roots of healthy trees or of trees cured by zinc treatment. This fact, together with the fact that something besides the reaction

FIG. 4.—Excellent growth of sunflowers in a soil on which peach trees will begin to die back in the first or second year in the orchard, if not treated with zinc.

of the soil and its supply of zinc influences the amount of zinc the top receives, suggested that some unfavorable condition in the soil renders the roots incapable of absorbing or of transporting zinc normally. Because zinc enters readily into organic combinations, it is possible that the condition in the roots which permits the leaching out of potassium might favor the combining with and holding of zinc by the roots. A few analyses suggested that zinc may accumu-

late in the roots. More extensive study, however, failed to support this suggestion.

Furthermore, experience in treating trees above ground, by spraying or by driving pieces of zinc into them, shows that without any change in the soil or in the supply of zinc to the roots of zinc deficient trees, these roots are capable of supporting at once a strong growth in the top. For example, in March, 1934, E. R. PARKER sprayed some grapefruit trees with zinc. The crop on the unsprayed trees was 56 pounds each; that on the sprayed trees, from blossoms that opened only a few weeks after the spraying, 477 pounds each, and the individual fruits were 66 per cent larger, all expressive of the greatly increased and healthy foliage. In one whole orchard, about 30 acres of mature Navel orange trees, the crop harvested the first autumn after a January spraying with zinc was said by the owner to be six times as large as the orchard had ever yielded before. About as striking results have followed dormant spraying of deciduous fruit trees with zinc sulphate (fig. 5). The strong healthy shoot growth follows from the first opening of buds in spring, instead of waiting upon improvement in root growth. There is little or no movement downward from points of treatment above ground. For example, when a branch is treated by driving pieces of zinc above some of its lateral branchlets, only the part above the zinc is cured. Branchlets below the zinc pieces still show zinc deficiency four years after those above the pieces have begun to show healthy growth. It seems certain that if much zinc had been moving downward from these pieces some of it would, within four years, have moved outward to these lower branchlets and have cured them. It seems that improvement in root growth after a treatment with zinc above ground is caused by improvement in foliage. In other words, for resumption of healthy growth and capable absorption, the roots require no other change than the substitution of healthy foliage for the weak, zinc deficient foliage.

SOIL ORGANISMS IN ZINC DEFICIENT ORCHARDS.—The soil flora seems to have some part in bringing on zinc deficiency. ARK (4) found that sterilization of the soil with steam, formalin, or ether caused plants to make as good growth as they made when treated with zinc sulphate. HOAGLAND (21) obtained similar results with

FIG. 5.—Cherry tree showing pronounced zinc deficiency symptoms. Most of the branches have died. The large leaves are on a shoot that had pieces of zinc driven into it early in the spring.

maize in this soil. After he sterilized the soil by autoclaving or with formalin, growth was as good as when he applied zinc sulphate to it. ARK (4) has isolated from soils on which trees showed pronounced zinc deficiency two strains of bacteria that, growing in the culture medium, cause maize, young apricot seedlings, and young walnut seedlings to show striking symptoms that are corrected by injecting zinc into the stems.

THEORIES TO EXPLAIN ZINC DEFICIENCY.—If all the symptoms that are cured with zinc are due merely to deficiency of zinc as an essential element, what causes the deficiency? All the facts presented might possibly be explained if there entered into the soil from the tree roots, or from manure in old corral areas, substances favorable to the ascendancy in the soil flora of species that absorb zinc in exceptional quantities, and if these species growing close to the root should take the zinc dissolved by acidity from the roots. Improved growth of plants in soils after sterilization might then be due partly to the fact that these organisms are no longer absorbing zinc and partly to the zinc supply released from them when they are disorganized. If the much larger supplies of nitrate nitrogen can be depleted by organisms when the supply of suitable carbon compounds in the soil is abundant, it does not seem unreasonable to suggest that, under conditions favorable to the selective growth of organisms that fix zinc, the minute quantities of zinc made available in the soil at the root surfaces may be so depleted.

The fact that zinc enters into many organic combinations suggests an alternative theory. The observations reported might possibly be explained if organisms such as those isolated by ARK excreted (or upon dying, yielded) substances that combined with zinc and held it insoluble. ARK, with ether and with alcohol, has extracted from a soil in which trees showed pronounced zinc deficiency symptoms, material that, poured into a culture medium, caused maize to show white-bud. He cured these symptoms by injecting zinc into the stem above the culture medium. It does not seem impossible that the heat of sterilization, the formalin, or the ether could break up such combinations and release the zinc.

I do not suggest a specific answer to the question as to how alfalfa among the trees enables them to obtain slightly more zinc, but it does not seem impossible that alfalfa roots could, by their acidity,

bring some zinc into solution, or by some influence on the soil flora, reduce the amount of zinc taken from the tree roots.

Some data seem difficult to harmonize with the view that all benefits from treatment with zinc are merely the result of supplying a deficiency. For example, in the second or third crop in the greenhouse on the soil HOAGLAND used, maize plants supplied with no zinc tend to make less growth than they should make from the zinc in the seed, less than they do make in special zinc free distilled water, yet plants treated with enough zinc sulphate will make normal growth. It might be held from such evidence as this that, both in water culture and in soil culture, zinc merely corrects something, perhaps some toxic substance from the microflora of the nutrient solution and of the soil. Much of what we are learning about responses of plants to zinc, however, does not seem to be explained by such a theory. The response of *Aspergillus* was in a sterilized medium. The responses of tree parts to treatment far above ground could be explained by such a theory only if the toxic substance were denatured when it reached the zinc after moving through the part below without injuring it. The injury in zinc deficient trees in spring and in late summer when symptoms are most striking is greatest in the uppermost parts. A toxic substance, therefore, would have to pass the fully open leaves and become concentrated enough to cause injury only in those just beginning to unfold. Other injurious substances, such as excess quantities of zinc or nitrogen compounds, often injure the lower older leaves, even cause them to fall, without injuring the young opening leaves. On the other hand, if the disease were due to lack of an essential element, it might be expected that the greatest injury would be in the top where the most growth is taking place. These facts, and many others, form a strong chain of evidence for zinc as an essential element and against the possibility of its merely denaturing toxic substances. Inconsistencies may be explained when we know more about the relation of the soil microflora to zinc and when we know more about the role of zinc in plants.

ROLE OF ZINC IN THE PLANT

There are 2000 to 3000 times as many nitrogen atoms as zinc atoms in tree tissue. Perhaps the proportion in other plants is simi-

lar. Workers have, therefore, generally assumed that zinc is not a permanent constituent of the compounds that form large parts of the protoplasm. The symptoms of zinc deficiency are not merely reduced growth without other striking injury, like those of nitrogen, phosphorus, and sulphur (**58, 59**), known to enter largely into the composition of important parts of the protoplasm; instead zinc deficiency seems to cause reduced growth only indirectly, by the injury it causes to foliage and young stems. Workers have generally suggested that it may catalyze some essential reaction. REED and DUFRENOY (**46**) think it may catalyze some part of the oxidation process in plants. The abundant growth that may accompany zinc deficiency injury, and the serious injury that precedes any considerable reduction in growth by such deficiency, would seem to suggest that zinc does not merely catalyze the release of energy for plant growth but may determine the direction of some process, such as oxidation, that without the influence of enough zinc may form injurious by-products.

If zinc should catalyze some process that is influenced by the carbohydrate supply, and more zinc were necessary with accumulation of carbohydrates, some of the behavior of zinc deficient plants might be explained; for example: the more severe injury on greenhouse plants in summer than during the short days in winter, the more severe injury on deciduous trees in spring and late summer when carbohydrates tend to be most abundant, the tendency of deciduous trees to improve in May and early June when carbohydrates are depleted by growth, and the more severe injury on Navel orange trees in the spring growth cycle following the accumulation of carbohydrates in winter (**9**).

If the role of zinc is that of a catalyzing agent, then it must catalyze some reaction that in the plant cannot be catalyzed by cadmium, mercury, silver, nickel, iron, cobalt, copper, manganese, chromium, boron, or titanium. When compounds of these elements (**12, 13**) were applied to branches of zinc deficient trees, through holes in the branches or otherwise, they failed to cause any improvement. There is conclusive evidence that they reached the buds. Compounds of tin, zirconium, tungsten, and molybdenum also caused no improvement; but since they produced no injury to show

that they had reached the twigs, it cannot be said with certainty that they were not all fixed in the wood before reaching the growing points.

Summary

1. There is strong evidence that zinc is an essential element for fungi and for higher plants; but, because of the very small amount of zinc required and its widespread presence as impurities, it has required exceptional methods to hold the zinc supply to plants in water cultures low enough to prevent moderate growth. Earlier workers were able to cause some improvement by supplying zinc, but some of them considered this response merely a stimulation, because the growth of check plants was approximately normal.

2. Widespread and serious injury to trees in orchards has been overcome by treating the trees with zinc, through the soil, by driving zinc or galvanized iron into the trunk or branches, and by spraying with zinc compounds on the foliage or on the dormant twigs.

3. The trees seem to take only about one ounce of zinc a year from an acre of soil, but under some conditions they are not able to obtain enough even for this small requirement. This is not always due to a small total zinc supply in the soil or to a reaction unfavorable to the solution of zinc. The soil flora seems to be involved: some soils in which plants show zinc deficiency will supply enough zinc after they have been sterilized. Theories are suggested to explain these phenomena.

4. The role of zinc in the plants is not known, but it is rather generally thought to act as a catalytic agent in some essential reaction.

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RELATION OF NUTRITION OF TOMATO TO DISPOSITION TO INFECTIVITY AND VIRULENCE OF FUSARIUM LYCOPERSICI

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 478

WILLIAM S. COOK

(WITH SEVEN FIGURES)

Introduction

The object of this investigation was to study the nutrition of the tomato as a factor in its disposition to infection by *Fusarium lycopersici*. The nutritional plane was varied through the supply of nitrates to the cultures kept under otherwise identical conditions. As the work progressed, a study of the browning of vascular bundles as the criterion of infection became essential.

Several investigators have studied the influence of fertilizers, or of some ingredients in the fertilizers, on infection by *F. lycopersici*, and the role of nutrition under controlled conditions of the substrate.

EDGERTON and MORELAND (5) found that the addition of large amounts of lime to the soil tended to hinder the rate of development of tomato wilt.

CLAYTON (3) found that plants grown in saturated soil were markedly different in their nitrogen relations from those grown in nonsaturated soil, as indicated by microchemical analysis. Resistance seemed to be directly correlated with the absence of nitrate nitrogen in the soils. He tested this relationship by growing plants in sand cultures and adding nutrient solutions. Part of the plants received a complete nutrient solution and the remainder a solution lacking nitrates. Temperature conditions in the greenhouse did not permit a virulent development of the disease, but "it was conclusively shown that plants grown without nitrate, the tissues of which plants give no nitrate test, are not infected by the fungus. Plants grown with a complete nutrient solution were readily infected" (p. 142).

AHMET (1) studied the influence of a varied mineral nutrient sup-

ply upon the pathogenicity of *Fusarium lycopersici*. His experiments were carried out under greenhouse conditions in sand cultures. Because he obtained 100 per cent infection, clear differences were apparent only in incubation time. The potassium deficient plants showed the shortest incubation time and the earliest appearance of wilt (17 days), with death of the plants in 25 days. Phosphorus deficient plants were next, with 22 and 30 day periods respectively. Excess nitrogen plants followed with an incubation period of 26 days, and with death of the plants in 37 days. Next in order were plants given excess phosphorus and excess potassium. Last were the nitrogen deficient plants, which died at the end of 58 days, even though they did not wilt.

FISHER (6) grew plants of the Bonnie Best (susceptible) and Marglobe (resistant) varieties in sand culture under deficient and excessive amounts of the essential elements. Boron and nitrogen deficiencies inhibited fungous infection in the Bonnie Best variety. Its susceptibility was decreased when the plants were treated with excesses of calcium, magnesium, and phosphorus, and solutions lacking in magnesium and phosphorus. The resistance of the Marglobe variety was decreased when the plants were treated with solutions lacking calcium and potassium and excessive in potassium, nitrogen, and sulphur.

EDGERTON (4) studied the development of tomato wilt in the susceptible Acme and the resistant Louisiana Wilt Resistant varieties in pot cultures. Different soils were used, including river sand, garden soil (loess), and two mixtures, one of soil sand and leaf mold and another of soil and leaf mold. The various soils were sterilized, heavily inoculated with *F. lycopersici*, and seeds of the two varieties planted therein. Infection results were based both on the number of plants dead from wilt and on those plants showing signs of the disease. In river sand, Acme gave 20.0 per cent infection while Louisiana Wilt Resistant gave only 3.1 per cent infection. In soil made up of a mixture of sand and leaf mold, the susceptible variety gave 92.9 per cent infection while the resistant variety gave 72.2 per cent.

EDGERTON and MORELAND (5) recognized a progressive yellowing and necrosis of leaves up the stem, leading finally to the wilted condition. CLAYTON (2) recognized a progressive wilting of the leaves,

often accompanied or preceded by a yellowing of the affected leaves. In well developed plants three to four weeks old, the first external evidence of the disease was always wilting of a lower leaf. Under conditions which were not favorable for the maximum development of the disease (resistant plants, fungus lacking in virulence, or environmental conditions unfavorable to growth of either parasite or host), there was a tendency for the disease to appear as a slow blight rather than as a wilt. In this blight the leaves yellowed and died slowly. AHMET found that young infections led, according to the conditions and degree of infections, either to a chronic disease course or to an acute one. Infection of grown plants led mostly to the acute disease course. He also recognized that death of the infected plants may come about without true wilting. He recognized yellowing and curling of leaves, stunting of plants, and bleaching of stems as additional *Fusarium* symptoms. FISHER gives a new, not previously reported, external disease symptom to tomato wilt. He states: "Adventitious roots occur on the stem which keep step with the yellowing of the leaves as the fungus spreads up the stem."

Material and procedure

FUNGUS CULTURES.—Cultures of *Fusarium lycopersici* used in the experiments were obtained from Dr. G. K. K. LINK of the Department of Botany, University of Chicago, and from Dr. F. L. WELLMAN of the United States Department of Agriculture. The culture furnished by Dr. LINK was used for the first experiment, but as it was thought that this culture was lacking somewhat in virulence, the latter culture was obtained and used in all of the other experiments. In pot tests made in soil with these two cultures side by side, it was found that the second culture was somewhat more virulent, although the first gave good results.

NUTRIENT SOLUTIONS.—The composition of the nutrient solutions used is given in table 1, which gives the partial volume molecular concentrations of the salts.

CULTURE OF PLANTS.—The investigation was conducted in the greenhouses of the University of Chicago. Seeds of Bonnie Best (susceptible) and Marglobe (resistant) were germinated in rich black potting soil. When they were about 5 cm. high the seedlings

were transplanted into flats containing soil, about 100 to the flat. When the plants were 7.5–12.0 cm. in height, the most vigorous were removed and transplanted into soil in 10 cm. clay pots, one plant per pot, where they were allowed to develop until ready for use. The time from planting of seed until satisfactory plants were obtained varied, being about six weeks to two months in the spring and summer and at least three months in the winter. When cuttings were used, the plants were trimmed to two leaves per plant, cut at the base just above the soil line (see the various experiments for deviations), immediately placed in buckets of water, and transplanted into the sand culture pots, two plants per pot. After transplanting, they were protected by newspapers from the sun for several days.

TABLE 1

COMPOSITION OF NUTRIENT SOLUTIONS

SOLUTION	$Ca(NO_3)_2$	$CaCl_2$	KH_2PO_4	$MgSO_4$
Plus nitrate	0.0090	.....	0.0045	0.0045
Minus nitrate	.....	0.0068	0.0045	0.0045

All of the experiments were carried out in sand cultures under controlled conditions, using soil-nutrient-temperature tanks (**8**). The aim was to grow the plants under environmental conditions most conducive to the type of growth desired. For the sand cultures, screened and sterilized white quartz sand was used; it was not subjected to any particular treatment of washing before use. An attempt was made to keep the sand below pH 6.0, and preferably at pH 5.5. Under such conditions the various salts are assimilated and the fungus grows readily. To attain this, all applications to the sand, either in the form of daily waterings with tap water or of nutrient solutions, were adjusted to pH 4.2.

Plants grown under plus nitrate culture received approximately 2.2 liters of plus nitrate solution per week, while those grown under minus nitrate culture received about 1.4 liters of minus nitrate solution during the same period. It was also necessary to flush with acidulated tap water once weekly in order to keep the concentration

of the salts in the sand below a possible toxic level. This aided in maintaining the desired acidity of the sand.

In all the experiments the temperature of the sand cultures was kept at 28° C. CLAYTON (**2**) found that this was the optimum temperature for growth of *Fusarium lycopersici*, and also very favorable for growth of the tomato. While he found that the air temperature was important in the development of the disease, the disease developing best under a soil temperature of 27° C. and an air temperature of 27° C., or equally as well under a soil temperature of 27° C. and an air temperature of 33° C., no attempt was made to control the air temperature in the greenhouses. The experiments extended through more than a year, with attendant variations in air temperature. Whenever possible air temperatures were kept at 21° C. during the night and at 25° C. during the day. Air temperatures up to 50° C. were recorded on some days.

INOCULATION OF PLANTS.—The ordinary plus nitrate nutrient solution in the several experiments served as an excellent growth medium for the *Fusarium* organism if supplemented with 3.3 per cent sucrose. In this solution an abundant growth was made in eight to ten days at ordinary room temperature. For cultural purposes, 250 cc. of the nutrient medium was placed in 500 cc. Erlenmeyer flasks, autoclaved, and later inoculated with a small amount of mycelium from a young culture of *F. lycopersici*.

The inoculum was either applied directly to the roots of the plants growing in sand (plants thus inoculated will be referred to hereafter as post-inoculated), or the sterile sand was inoculated previous to planting (plants referred to hereafter as pre-inoculated). In the former method the sand was pulled back from around the roots to the depth of 10–12 cm. and the contents of one culture flask (250 cc.) poured over the roots. In the latter method about 20 cm. of the sterilized sand was removed from the soil containers, the contents of a single culture poured in, and the removed sand replaced and mixed thoroughly with the inoculum. At various times during the experiments, isolations were made from the sand cultures to check the presence of the fungus. Results of these isolations showed that the organism grew well in the sand without the addition of carbohydrates.

SEEDLING TESTS.—Germination of seed and growth of seedlings took place under plus and minus nitrate nutrition. After germination, the seedlings were thinned to a definite number per pot. Infection results are based on the recovery of the fungus from any part of the stems of the seedlings. All isolations which showed fungous growth were checked microscopically.

CRITERIA OF INFECTION.—The first experiment revealed that neither the acute nor the chronic disease picture described by AHMET is an adequate diagnostic test of invasion by *Fusarium lycopersici* and of its establishment in the tomato plant. Microscopic examination of stems and plate cultures also showed that vascular browning of the stems alone, as found by FISHER, is not an invariant test. Consequently recovery of the fungus from the base of the stems, 3–5 cm. above the sand line, was used as a criterion of infection. All isolations which showed fungous growth were studied microscopically to ensure identification of *F. lycopersici*. In many cases isolations were made from the middle and top of the stem.

Experimental results

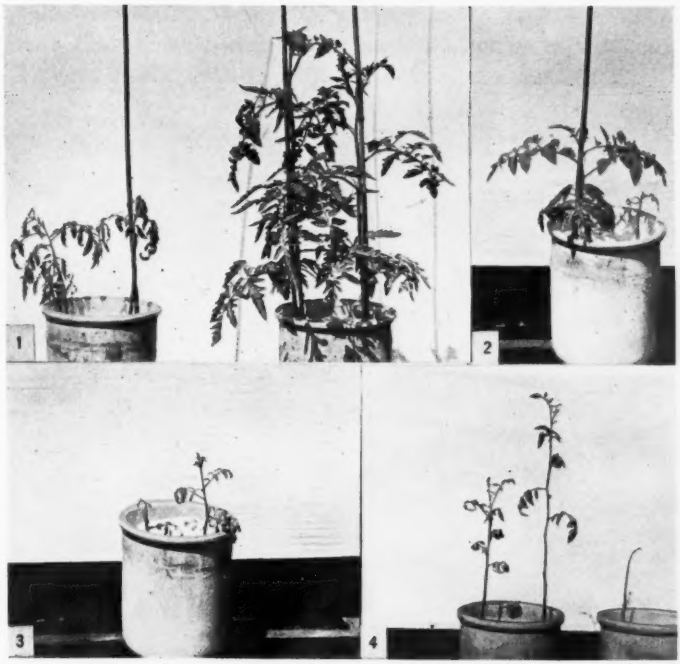
SYMPTOMS OF THE DISEASE

It was found that under the experimental conditions imposed, wilting was not the most characteristic symptom of tomato plants invaded by *F. lycopersici*, and that it may be completely absent in plants that die as a result of a slow necrosis of parts.

As pointed out by AHMET, two general disease pictures may be recognized: (a) the "chronic" type (figs. 1–4) in which the disease course is slow, and (b) the "acute" type (fig. 5) in which the disease course is fairly rapid, with true wilting.

Disease symptoms appearing in the plus nitrate plants were of both the chronic and the acute type, and were abundant in the susceptible plants. Resistant plants as a whole developed few symptoms, these being of the chronic type (fig. 2). True wilt was not observed in the resistant plants.

Infected minus nitrate (high carbohydrate) plants were characterized by the chronic type of disease picture (figs. 3, 4). Symptoms were harder to follow, however, owing to the fact that yellowing and abscission of leaves was constantly taking place in the uninoculated



FIGS. 1–4.—Fig. 1, susceptible Bonnie Best, plus nitrate plants, from experiment IV. Two plants with chronic type of disease shown in culture pot on left. Note absence of lower leaves in righthand plant, also stunted condition of both plants. Control plants in pot at right. Plants under nutritional treatment since April 13, 1936. Photographed May 22. Fig. 2, resistant Marglobe variety under plus nitrate nutrition. Plant at right manifests chronic type of disease and is extremely stunted; lefthand plant apparently healthy but gave, at end of experiment (July 2, 1936), fungus on culturing. Experiment, date of photograph, and cultural conditions same as for fig. 1. Fig. 3, Bonnie Best variety under minus nitrate culture; experiment IV. Plant at left shows chronic type of disease and is stunted; plant at right apparently healthy but gave positive recovery of fungus when cultured at end of experiment. Plants under minus nitrate culture since April 13, 1936. Photographed May 22. Fig. 4, Bonnie Best variety under minus nitrate culture; experiment IV. Plant in righthand pot manifests chronic type of disease symptoms, is stunted, and has lost all leaves except topmost one. Control plants in pot on left. Plants under minus nitrate culture since April 13, 1936. Plants photographed at same time as those shown in fig. 5.

plants. Symptoms of the diseased plants could be recognized, however, for in the uninoculated plants the tops remained green; whereas, in the diseased ones, yellowing involved all of the leaves, until the

FIGS. 5, 6.—Fig. 5 (left), Bonnie Best, plus nitrate plants, from experiment IV. Righthand plant, center pot, shows acute type of disease picture, with definite wilting; lefthand plant, yellowing and necrosis of leaves approximately half way up the stem. Culture pot on right shows two diseased plants which as yet show no wilting. Control plants in culture pot on left. Plants under nutritional treatment since April 13, 1936. Photographed June 3. Fig. 6 (right), representative control plants of experiment I showing effects of plus and minus nitrate nutrition on vegetative growth of tomato plants. Plus nitrate plants in culture pot on left, minus nitrate (high carbohydrate) plants on right. Photographed July 1, 1935.

plant was finally denuded of all foliage (fig. 4). Stunting of the plants was usually another definite symptom which could be used to differentiate between healthy and diseased plants (figs. 3, 4). In a few cases some of the plants showing the chronic type of symptoms recovered. Both resistant and susceptible minus nitrate plants manifested the same chronic type of disease picture.

In addition to the regular chronic and acute symptoms noted, additional ones were observed. Cessation of growth in the apical meristems (stunting), already mentioned, was a striking feature, and in most cases of regular occurrence (figs. 1–4). In fact growth measurements made at weekly intervals often identified diseased plants before other symptoms were evident. Another striking symptom occurring in many cases as a part of the chronic disease picture, and only in the plus nitrate plants, was that of the intense greening of the topmost leaves. Plants in this stage presented a striking appearance. They were stunted, with the intensely dark green leaves flaccid and slightly drooping but not wilted. This incipient flaccidity of the leaves sometimes (but not usually) developed into a true wilt, death of the leaves coming about through slow necrosis.

In connection with some fumigation in the greenhouses, plants in experiment IV were given two applications of cyanide to control the white fly. After both fumigations it was noticed that the upper leaves were severely burned. The leaves of those plants which showed definite *Fusarium* symptoms (plus nitrate plants), however, were entirely free from cyanide injury. No attempt has been made to explain this observation. Possibly the explanation lies in AHMET'S observations that the rate of transpiration in infected tomato plants was much less than in healthy ones, and that in addition, the diseased plants had their stomata closed.

Plants in experiment IV were checked for the presence or absence of adventitious roots, relative to FISHER'S observation that such roots occur on the stems of diseased tomato plants. His observations were found to be diagnostic for plus but not for minus nitrate plants. Adventitious roots have also been noted on diseased petioles of plus nitrate plants.

BROWNING OF VASCULAR BUNDLES AS CRITERION OF INFECTION

Various investigators have noted definite browning of the vascular bundles of stems of affected plants and many have used it as a criterion of infection. In the course of the different experiments, observations were made on the presence or absence of vascular browning at the base of the stems. An attempt has been made to

correlate these results with the infection results, which are based on the isolation of the fungus from the stem base.

Results given in table 2 show that out of a total of 275 plants, 144 gave definite browning of the vascular bundles, with 138 (95.8 per cent) positively infected, and 131 gave no evidence of brown vascu-

TABLE 2

BROWNING AND NON-BROWNING OF VASCULAR BUNDLES AS CRITERION OF INFECTION

MATERIAL	CONDITION OF VASCULAR BUNDLES IN RELATION TO INFECTION									
	TOTAL NO. PLANTS	BROWN BUNDLES AT BASE			NO BROWN BUNDLES AT BASE			TOTAL INFECTED		
		No.	NUMBER INFECTED*	PERCENTAGE INFECTED*	No.	NUMBER INFECTED	PERCENTAGE INFECTED	No.	PERCENTAGE WITH BROWN BUNDLES	PERCENTAGE WITH NO BROWN BUNDLES
Bonnie Best + nitrate	69	54	52	96.3	15	5	33.3	57	91.2	8.8
Marglobe + nitrate	70	36	33	91.7	34	4	11.8	37	89.0	11.0
Total	139	90	85	.....	49	9	.....	94	.....	.....
Average	.....	.....	.....	94.4	....	.....	18.3	....	90.4	9.6
Bonnie Best − nitrate	66	31	31	100.0	35	17	48.6	48	64.6	35.4
Marglobe − nitrate	70	23	22	95.6	47	12	25.5	34	64.7	35.3
Total	136	54	53	.....	82	29	.....	82	.....	.....
Average	.....	.....	.....	98.1	....	.....	35.4	....	64.6	35.4
Grand total	275	144	138	.....	131	38	.....	176	.....	.....
Average	.....	.....	.....	95.8	....	.....	29.0	....	78.4	21.6

* Infected means presence of fungus in tissues of host.

lar bundles. Of these, 38 (29.0 per cent) were infected, however; that is, they yielded fungus upon culturing. One hundred seventy-six infected brown bundle plants and infected non-brown bundle plants were found; 78.4 per cent of these had brown bundles, and 21.6 per cent, no brown bundles. A comparison of the amount of bundle browning and the amount of infection resulting shows no ap-

preciable differences, either for the two varieties or for the different kinds of nutrition. Practically all (95.8 per cent) of the isolations from the base of the stems with brown bundles gave positive infections.

In those plants showing no brown bundles at the base, however, results are different. Minus nitrate plants showed high infection (35.4 per cent), while in the plus nitrate plants only 18.3 per cent of the plants with no browning of vascular bundles were infected.

Nutritional experiments

Experiment 1 (table 3)

On March 29, 1935, cuttings of Bonnie Best and Marglobe varieties of tomatoes were transplanted into sterilized sand. The plants were fairly succulent and gave a distinct test for nitrates. Five days after transplanting, all cuttings received plus nitrate solution and were grown continuously under plus nitrate culture for the next 24 days (period ending April 22). At the end of this period approximately half of the plants of each variety were transferred to minus nitrate culture. At the time of changing cultural conditions the average height of all plants was 23.1 cm.

On May 7, all plants were inoculated by pouring fungous inoculum around the roots. At the time of inoculation the plus nitrate plants (especially the tops) were growing vigorously and were fairly succulent. The stems were large and stocky, with an average height of about 53.5 cm. In contrast to the plus nitrate plants, the minus nitrate plants were much less vegetative and succulent, the lower leaves beginning to turn yellow, shrivel, and absciss. The stems were hard, yellowish green in color, and of small diameter. These plants were becoming typical, hard, high carbohydrate plants; they averaged 45.4 cm. in height (fig. 6).

During the elapsed period, from the time of inoculations (May 7) to the end of the experiment (August 1), all plants which exhibited definite *Fusarium* symptoms were removed and used for petri dish isolations. At the end of the experiment (72 days from time of inoculation to removal) all plants were cut off about 5 cm. above the surface of the sand, measured, descriptive notes taken, and they were then brought into the laboratory for isolation studies. In all

TABLE 3

Material	Controls		Inoculated plants																
				With external symptoms*					Without external symptoms†						Not infected§		Total infection‡	
							Infected‡					Infected‡						
	Number	Average height (cm.)	Total no. plants	Number	Percentage of total	Average height (cm.)	Number	Percentage	Number	Percentage of total	Average height (cm.)	Number	Percentage	Average height (cm.)	Number	Average height (cm.)	Number	Percentage
	No. I, post-inoculated,‖ May 7–August 1																	
Bonnie Best + nitrate..	6	182.3	24	4	16.7	132.7	4	100.0	20	83.3	201.0	14	70.0	210.0	6	177.0	18	75.0
Bonnie Best − nitrate..	5	86.0	24	0	.....	.....	0	.....	24	100.0	85.4	15	62.5	84.7	9	86.6	15	62.5
Marglobe + nitrate....	6	181.3	24	0	.....	.....	0	.....	24	100.0	187.0	1	4.0	157.0	23	188.3	1	4.0
Marglobe − nitrate....	5	105.0	24	0	.....	.....	0	.....	24	100.0	91.0	11	45.8	88.5	13	93.2	11	45.8
	No. II, post-inoculated,‖ August 15–October 25																	
Bonnie Best + nitrate..	4	222.0	12	8	66.7	99.4	8	100.0	4	33.3	221.5	3	75.0	213.3	1	246.0	11	91.7
Bonnie Best − nitrate..	2	50.5	9	0	.....	.....	0	.....	9	100.0	52.8	7	77.8	52.4	2	54.0	7	77.8
Marglobe + nitrate....	4	206.0	12	0	.....	.....	0	.....	12	100.0	228.7	8	66.7	224.6	4	234.3	8	66.7
Marglobe − nitrate....	2	40.5	12	0	.....	.....	0	.....	12	100.0	50.7	1	8.3	37.0	11	51.9	1	8.3

* Characteristic external symptoms of Fusarium injury.

† Apparently healthy plants without externally detectable Fusarium injury, but not without vascular browning.

‡ Infected means presence of fungus in tissues of host.

§ Not infected means no fungus recoverable by culture on agar plate.

‖ Substrate inoculated after transplanted plants had rooted.

TABLE 3—*Continued*

Material	Controls		Inoculated plants															
				With external symptoms*					Without external symptoms†						Not infected§		Total infection‡	
							Infected‡					Infected‡						
	Number	Average height (cm.)	Total no. plants	Number	Percentage of total	Average height (cm.)	Number	Percentage	Number	Percentage of total	Average height (cm.)	Number	Percentage	Average height (cm.)	Number	Average height (cm.)	Number	Percentage
	No. III, pre-inoculated,¶ October 18–January 10																	
Bonnie Best + nitrate	3	131.0	9	5	55.6	19.2	5	100.0	4	44.4	138.0	1	25.0	143.0	3	133.7	6	66.7
Bonnie Best − nitrate	2	59.0	9	4	44.5	15.0	0	.....	5	55.5	36.2	0	.....	.....	5	36.2	0	.....
Marglobe + nitrate	3	125.0	10	0	.....	.....	0	.....	10	100.0	119.8	7	70.0	118.4	3	123.0	7	70.0
Marglobe − nitrate	2	36.0	10	1	10.0	27.0	0	.....	9	90.0	38.1	1	11.1	48.0	8	36.9	1	10.0
	No. IV, pre-inoculated,¶ April 13–July 2																	
Bonnie Best + nitrate	6	111.0	24	15	62.5	42.6	14	93.6	9	37.5	93.9	8	88.9	90.1	1	124.0	22	91.7
Bonnie Best − nitrate	6	30.9	24	15	62.5	4.5	15	100.0	9	37.5	22.9	9	100.0	22.9	0	.....	24	100.0
Marglobe + nitrate	6	100.8	24	7	29.3	41.4	7	100.0	17	70.7	78.3	14	82.5	75.3	3	92.7	21	87.5
Marglobe − nitrate	6	24.3	24	10	41.7	2.9	9	90.0	14	58.3	15.7	12	85.7	16.3	2	12.3	21	87.5

¶ Substrate inoculated prior to transplanting of plants.

cases isolations were made from the bottom of the stem; in some instances from the middle and the top as well. All isolations were incubated at 28° C., and studied microscopically.

EXPERIMENT II (TABLE 3)

Starting July 15, 1935, cuttings of Bonnie Best and Marglobe were transplanted into sterilized sand. The cuttings were from plants growing in soil in flats. Owing to the crowded condition in the flats, the plants were spindly (approximately 45 cm. in height), with most of their lower leaves abscissed. The upper portions of the plants were vegetative and fairly succulent. For the cuttings, only the upper 25 cm. of the plants was used. About a week after transplanting, the individuals of each variety were divided into approximately two equal lots, one lot of each variety being grown under plus nitrate and the other under minus nitrate culture. The set-up of this experiment varied from the preceding one, in that the experimental plants, which were to be used later for the high carbohydrate, minus nitrate plants, received no nitrates after being transplanted. For the next 30 days (July 15 to August 15) all plants were grown under the cultural conditions already outlined.

Starting August 15, all plants were inoculated as in experiment I (post-inoculated). The plus nitrate plants averaged 48.9 cm. in height, were vegetative, green, and very succulent; the minus nitrate plants averaged 20.1 cm. in height, were spindly, with woody stems and small lower leaves that were turning yellow and beginning to abscis, and with light green and small top leaves. In appearance these plants were typically high carbohydrate (fig. 7).

From the time of inoculation to the end of the experiment (72 days, ending October 25), the plants were carefully watched for symptoms of *Fusarium* damage. At the end of the experiment all plants were removed for isolation studies. In all cases isolations were made from the bottom of the stem, and in this experiment from the middle and top of the plant as well. All fungous growth was checked microscopically.

EXPERIMENT III (TABLE 3)

This experiment and the following differ from the preceding in that cuttings were transplanted directly into sand previously inocu-

lated with *F. lycopersici* (pre-inoculation). This procedure shortened the incubation period.

Sand was inoculated on August 23, 1935, and the fungus allowed to incubate until October 18 (55 days). During this period the inoculated sand was watered once daily with tap water, and in addition, each of the inoculated pots received one quart of plus nitrate nutrient solution at weekly intervals.

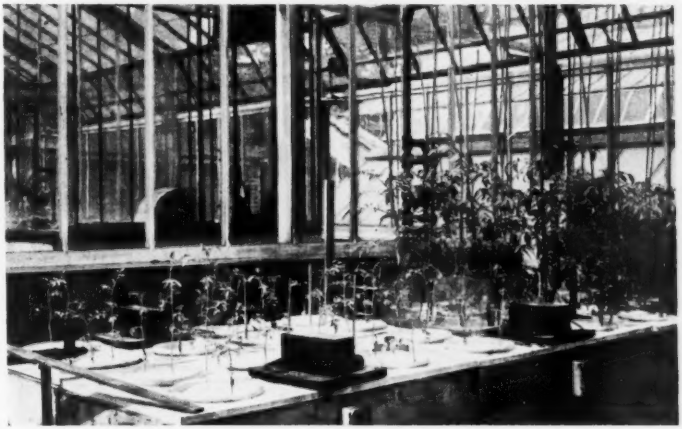


FIG. 7.—Growth of tomato grown in plus and minus nitrate culture (experiment II). Plus nitrate plants on right, minus nitrate plants on left. Plants under nutritional treatment since July 15, 1935. Photographed August 19.

On October 18, cuttings of Bonnie Best and Marglobe were transplanted into the inoculated sand. Plants used as cuttings for the minus nitrate cultures averaged about 45 cm. in height, and were fairly hard near the base. In making these cuttings only the upper 25 cm. of the plant was used. Plants used in the plus nitrate cultures averaged 25–30 cm. in height, were younger than those used in the minus nitrate pots, and more succulent and vegetative. About one week after transplanting, the plants of each variety were divided into approximately two equal lots, one of which was grown under plus nitrate and the other under minus nitrate culture. In this experiment, as in experiment II, plants growing under minus nitrate culture received no nitrates after transplanting; after which the

plants grew well. Those receiving nitrates became typical plus nitrate plants while those receiving no nitrates became typical high carbohydrate plants.

Plants were carefully watched for *Fusarium* symptoms from the time of inoculation (October 18) to end of the experiment (84 days, ending January 10, 1936). As in previous experiments, isolations were made from the bottom of the stems in all cases, and in this experiment mostly from the middle also; a few isolations were made from the top of the stem as well.

Experiment IV (table 3)

As in experiment III, cuttings were transplanted directly into inoculated sand.

The sand was inoculated on April 2, 1936, and the fungus allowed to incubate in it for 12 days. During this period all pots containing the fungus were watered once daily with tap water, and in addition each pot received two quarts of plus nitrate nutrient solution.

On April 13, cuttings of Bonnie Best and Marglobe were transplanted into the inoculated sand. Plants used for cuttings were growing in small pots and averaged 20–25 cm. in height. These plants were highly vegetative and succulent, except near the base. They were blooming, and the bottom leaves were beginning to turn yellow and absciss. They gave a distinct test for nitrates and a rather weak test for starch. Plants were prepared for cuttings in the usual manner. Five days after transplanting, the plants of each variety were divided into two equal lots, one-half of each lot being grown under plus nitrate and the other under minus nitrate culture. Plants grown under minus nitrate culture received no nitrates after transplanting; after which the plants made good growth.

Plants were carefully checked for *Fusarium* symptoms from the time of inoculation to the end of the experiment (79 days, April 13 to July 2). Those plants which showed definite enough symptoms to warrant a tentative diagnosis of the disease were removed during the course of the experiment and used for isolation studies. At the end of the experimental period, all remaining plants were removed and used for isolations from the base of the stem. In addition, isola-

tions were made from the middle and top of the stem as well for most plants.

Results obtained from experiments I to IV show the Bonnie Best (susceptible) plus nitrate plants to be highly infected under both types of inoculation at all seasons of the year. The average infection for all plants in the four experiments amounted to 82.6 per cent; 46.4 per cent displayed symptoms.

Infection in the Marglobe (resistant) variety, under plus nitrate culture, also was high in all except experiment I, in which only 4.0 per cent infection was noted. While an average infection of 52.9 per cent was noted in all experiments, only 4.0 per cent of the plants in experiment I were infected. Only 10.0 per cent of all the inoculated plants showed symptoms, all of which were obtained in experiment IV.

Under minus nitrate culture, the percentage of infection was high for the Bonnie Best variety in all except experiment III, which gave no infection at all. The average infection for all of the inoculated plants was 69.7 per cent. Of the post-inoculated (experiments I and II) plants, 66.7 per cent were infected, while 75.8 per cent of the pre-inoculated (experiments III and IV) were infected. Only 19 plants (28.8 per cent) of all the inoculated plants showed symptoms, 15 of which occurred in experiment IV. In experiment III, four plants out of a total of nine exhibited symptoms, but did not yield the fungus when cultured.

Of inoculated plants of minus nitrate Marglobe variety, 48.6 per cent were infected. In experiment I, with post-inoculated plants, 45.8 per cent were infected. In experiment IV, with pre-inoculated plants, 87.5 per cent were infected. In experiments II and III, an average infection of only 9.1 per cent was obtained. In experiment II the plants were post-inoculated and the period extended from August to October, while in experiment III the plants were pre-inoculated, the period extending from October to January. Production of symptoms was low in these plants; only 11 (15.7 per cent) of the inoculated plants developed symptoms, with all but 1.4 per cent occurring in experiment IV. Two plants, one each from experiments III and IV, exhibited symptoms but were negative when cultured.

An average incubation time of 44 days was noted in the post-in-

oculated plants, while an average incubation time of 25.5 days was noted in the experiments in which the plants were put directly into inoculated sand.

SEEDLING TESTS (TABLE 4)

On October 19, 1935, seeds of Bonnie Best and Marglobe were placed in sand which had previously been inoculated, on August 23. From the time of planting to the end of the experiment, lots of each

TABLE 4

INFECTIVITY OF FUSARIUM LYCOPERSICI TO BONNIE BEST (SUSCEPTIBLE) AND MARGLOBE (RESISTANT) TOMATO SEEDLINGS GROWN IN INOCULATED SAND UNDER PLUS NITRATE AND MINUS NITRATE CULTURE

MATERIAL	INOCULATED PLANTS											
	TOTAL NO. PLANTS	WITH SYMPTOMS				WITHOUT SYMPTOMS						TOTAL INFECTION
		NO.	PERCENTAGE	INFECTED*		NO.	PERCENTAGE	INFECTED*		NOT INFECTED†		
				NO.	PERCENTAGE			NO.	PERCENTAGE	NO.	PERCENTAGE	NO.	PERCENTAGE
Bonnie Best + nitrate	55	19	34.5	5	26.3	36	65.5	3	8.3	33	91.7	8	14.5
Bonnie Best − nitrate	40	25	62.5	16	64.0	15	37.5	2	13.3	13	86.7	19	47.5
Marglobe + nitrate	63	30	47.6	20	66.7	33	52.4	7	21.2	26	78.8	27	42.8
Marglobe − nitrate	74	21	28.4	13	61.9	53	71.6	1	1.9	52	98.1	14	18.9

* Infected means presence of fungus in tissues of host.

† Not infected means no fungus recoverable by culture on agar plates.

variety were grown in plus as well as in minus nitrate culture, the latter receiving no nitrates during the experiment.

Seeds of both varieties germinated well under the two types of nutrition. Following germination, the seedlings made good growth, the plus nitrate ones being the larger. On November 5 the seedlings were thinned to a uniform distribution in the culture pots. Seedlings were watched daily for *Fusarium* symptoms, and plants showing such symptoms were removed and cultured.

Healthy plus nitrate seedlings made the greater growth. The plus nitrate seedlings were taller, averaged 4.1 cm. in height, with about three true leaves. They were very succulent, with weak stems, and had a tendency to fall over. On the other hand the minus nitrate plants were stiffer and stood up much better, averaged 2.6 cm. in height, and usually produced only one true leaf. The experiment was terminated during the period from December 7 to 10, and all remaining seedlings were removed and used for isolations.

Of the inoculated Bonnie Best plus nitrate plants, 14.5 per cent were infected, with 34.5 per cent developing symptoms.

Of the inoculated Marglobe plus nitrate plants, 42.8 per cent were infected, with 47.6 per cent of the seedlings showing symptoms.

In the Bonnie Best minus nitrate inoculated plants, total infection amounted to 47.5 per cent, with 62.5 per cent of the inoculated individuals showing symptoms.

The Marglobe minus nitrate inoculated seedlings gave a total infection of only 18.9 per cent, 28.4 per cent of the inoculated individuals producing symptoms.

Discussion

It is apparent from the data obtained in these experiments that the so-called susceptibility and resistance of the tomato to *Fusarium* wilt has at least two aspects. One of these is a positive or a negative disposition to infection, that is, to entry of the parasite into the host and its subsequent establishment and spread; the other, a positive or a negative disposition on the part of the tomato to manifest the structural, processal, and behavioral symptoms characteristic of the disease. The former aspect is a measure of the infectivity or noninfectivity of the parasite; the latter of its virulence or avirulence (7).

It is also evident from the results of these experiments that disposition of the tomato to manifest disease symptoms is not unitary. Some infected plants show both vascular discoloration and other symptoms. Others show neither vascular discoloration nor any other disease symptom. Therefore browning of vascular tissues, frequently used as a criterion, is not a measure of the disposition of the tomato to infection.

Because these two aspects of disposition are not clearly differen-

tiated, and because the term infection is indiscriminately used to designate the disease, or some manifestation of symptoms, as well as the state of being contaminated (7), it is difficult to interpret much of the literature dealing with tomato wilt and other wilt diseases.

For the experiments reported here, infection means the state of being contaminated by *Fusarium lycopersici*. It means that the fungus has entered the host, established itself, and spread from the original site of establishment. It does not mean wilt disease in its usual sense. Possibly infection so interpreted involves some injury to cells, so that considered at the cellular level of biological organization, infection of tomato by *F. lycopersici* probably is characterized by microscopically detectable injury and injury reactions; that is, by disease.

The experimental data show that the expression of each of the two aspects of disposition of the tomato is affected by nutrition in both the so-called susceptible and resistant varieties.

CLAYTON (3) states that susceptible varieties of tomatoes grown under minus nitrate conditions "were not infected." If by this he means the fungus was not recoverable in culture, the results of this investigation are at variance with his, because susceptible Bonnie Best plants grown under minus nitrate nutrition yielded fungus upon culturing, and also developed symptoms in one experiment (IV). These results also are at variance with FISHER'S (VI), who used browning as a criterion of susceptibility of infection, and reports that the Bonnie Best variety was characterized by lack of infection when grown in absence of nitrates.

FISHER reports that the resistant Marglobe showed practically no infection when grown in absence of nitrates. The results of this study are at variance with his, in that in two experiments (I and IV) Marglobe showed infection when grown without nitrates. FISHER reports that Marglobe developed infection when grown with an excess of nitrates. In the experiment reported here, frequency of infection was high in both the resistant and susceptible varieties (except in experiment I) when grown in plus nitrate nutrition. In experiment I Marglobe was resistant to infection.

The writer is unable to advance any plausible explanation for the exceptions noted in experiments I–IV. Average frequency of infec-

tion of Marglobe plants was higher when the plants were transplanted into pre-inoculated sand (experiments III and IV) than when inoculation was effected by working the fungus into the sand around established plants (experiments I and II). Possibly severe trauma and other injuries incident to cutting and transplanting disposed the plants to infection. Bonnie Best also showed a higher average frequency of infection in the experiments (III and IV) involving cutting and transplanting into previously inoculated sand. It is possible that the amount of fungus present was greater and more effective in the pre-inoculated sand experiments. Possibly the age of the plants also contributed to injury. These transplanted plants were younger and more succulent at the base than the established plants. Possibly differences in air temperature, humidity, and sunshine were factors, through different influences on the nutritional state of the experimental plants at different seasons of the year. Generally speaking, both plus and minus nitrate plants contain more carbohydrates (starch) during the late spring, summer, and early fall months than during the winter months. Plus nitrate plants are high in nitrates at all seasons of the year, while minus nitrate plants contain no readily demonstrable nitrates at any season of the year.

I am also not prepared to explain the differences in frequency of expression of symptoms noted in experiments I–IV. Only Bonnie Best plants when post-inoculated were disposed to symptom manifestation in plus nitrate culture. In pre-inoculation experiments, however, both Bonnie Best (in experiments III and IV) and Marglobe (in experiment IV) were disposed to symptom manifestation in plus nitrate culture. The former alone manifested true wilting in addition to the chronic type of symptoms; the latter manifested only the chronic type. The same factors that were responsible for irregularities in the infection data may have been responsible for deviations in the manifestation of symptoms. Pre-inoculations and post-inoculations also affected the incubation time, symptoms appearing in 25 days for the first and in 44 days for the latter.

These experiments reveal discrepancies between frequency of browning in the stem or manifestation of other symptoms, and between frequency of recovery of the fungus by culture. Plants with brown bundles (experiments III and IV) did not invariably yield the

fungus in culture. This does not mean that they were not infected, because presence or absence of the fungus in the root system was not determined by culture. It is well known that browning may occur in advance of the site of the fungus. A decisive answer to this problem would entail culture of the entire root system of every plant.

The other discrepancy, namely the recovery of fungus from plants showing no symptoms whatsoever in the aerial parts, indicates manifestation of low virulence on the part of the parasite and of high resistance to virulence on the part of the plant. This discrepancy was most marked in plants under minus nitrate nutrition.

Possibly the discrepancy between infection as determined by culture on the one hand and by browning on the other would have been less if microscopically detectable browning had been used as a criterion of presence of the fungus. FISHER used the microscopic criterion.

Summary

1. Experiments were conducted to study the relation of nutrition of the tomato as a factor in its disposition to the infectivity and virulence of *Fusarium lycopersici*. Bonnie Best (susceptible) and Marglobe (resistant) varieties were tested. Nutrition was varied by the application or non-application of nitrates under otherwise identical conditions. The substrate was inoculated both previous (pre-inoculation) to and subsequent (post-inoculation) to the establishment of the plants.

2. Recovery of the fungus from the base of the stem was used as a criterion of infectivity and infection. Manifestation of macroscopically detectable symptoms was used as a criterion of virulence and of pathic effects in the host.

3. Results of the nutritional experiments revealed (a) a high frequency of infection of both resistant and susceptible tomato plants under minus nitrate nutrition; (b) a low frequency of symptoms in plants under minus nitrate nutrition; (c) no wilting in the so-called resistant Marglobe under either type of nutrition; (d) seedlings of the resistant variety under either plus or minus nitrate culture read-

ily infected and producing symptoms identical with those of the susceptible variety.

4. Type of inoculation and age of the plant are important factors in infection and symptom production: (a) Pre-inoculation of sand led to a higher frequency of infection and symptom production. (b) Post-inoculation of sand under minus nitrate culture did not lead to the development of any plant symptoms, even in infected plants. (c) Bonnie Best variety under plus nitrate nutrition showed a high frequency of infection and of symptom production of both the chronic and the acute type, at all seasons of the year, when either pre-inoculation or post-inoculation was used. (d) Pre-inoculation practically halved the incubation period.

5. Browning of vascular bundles of the stem alone is an inadequate criterion of infection. Even under minus nitrate nutrition, 35.4 per cent of the infected plants did not manifest brown bundles at the base of the stem.

The writer gratefully acknowledges the valuable assistance and criticisms of members of the staff of the Botany Department at the University of Chicago during the progress of this work.

TALLULAH, LOUISIANA

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EQUIPMENT FOR GROWING PLANTS IN NITROGEN FIXATION STUDIES

C. A. LUDWIG

(WITH THREE FIGURES)

Introduction

In certain nitrogen fixation and related studies in this laboratory during the last five or six years (**1, 14, 15, 16**), the writer has been using two kinds of apparatus for growing the plants in artificial light. The first type of apparatus produces good growth of plants in considerable numbers under conditions which are rather closely reproducible. However, many of the refinements of control necessary when study of the light relations of plants is undertaken have been omitted, since the purpose was merely to grow plants in fairly large numbers with some approach to "normal" characteristics. The apparatus is easily constructed, inexpensive, and requires little attention in operation. A second type of apparatus has been used when it was desirable to grow plants under more rigidly controlled biological or atmospheric conditions. The following description of the equipment concerned has been prepared in response to the suggestion that it would prove helpful to others with a similar problem.

Apparatus no. 1

The apparatus, the essential structural details and the general appearance of which are shown in figures 1 and 2, differs from previously described installations chiefly in that all types of light filters, air conditioning devices, etc., for improving the spectral balance of the light and for preventing high air temperature and aridity have been discarded. Instead some mercury vapor lamps have been added to improve the spectral balance and a water bath has been introduced to keep root temperatures from becoming too high. The apparatus consists of a table supporting the water bath, in which the culture jars are placed, an overhead bank of lights, and the necessary accessory equipment for operation.

The water bath is a copper lined wooden tank. The water enters from the city water system through small holes drilled in a pipe which lies on the bottom of the tank and against the wall on three sides. It leaves through an overflow pipe which can be adjusted as to height so as to give within limits any desired depth of water. The temperature and rate of admission of the cold water determine the

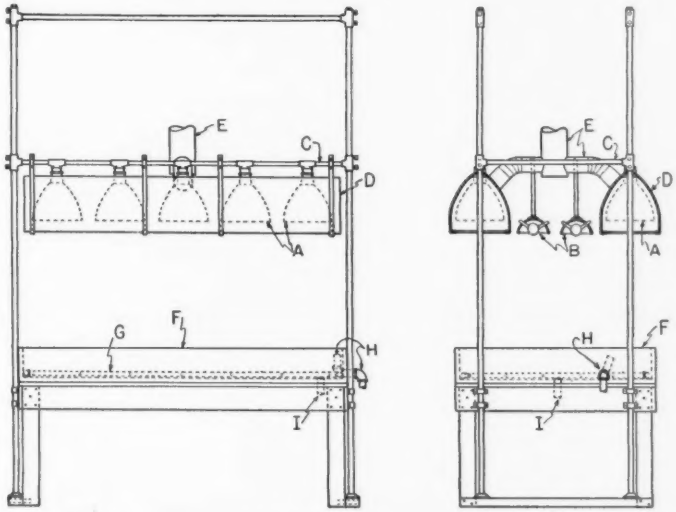
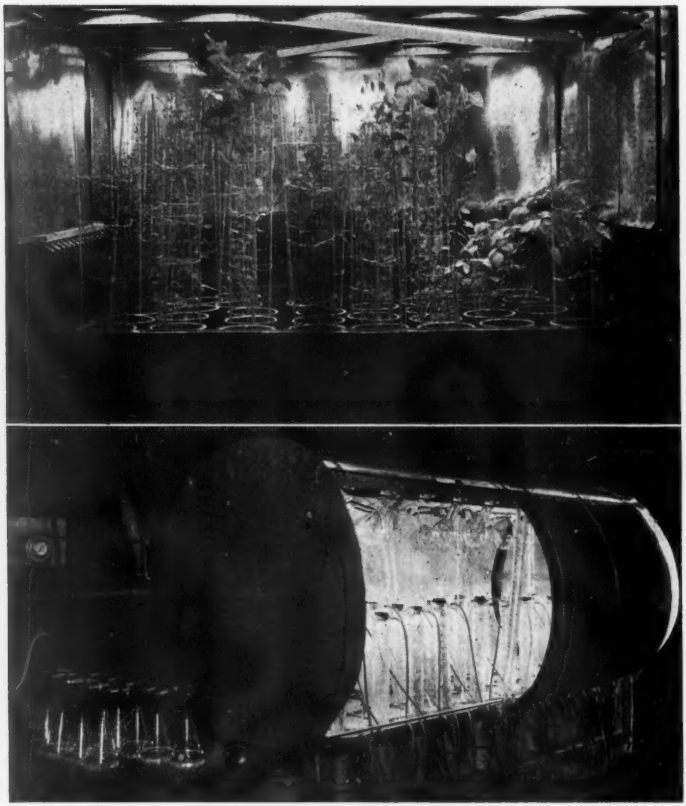


FIG. 1.—Drawing of side and end of plant culture apparatus: *A*, enameled reflectors; *B*, mercury vapor lamps; *C*, adjustable framework supporting the lamps; *D*, galvanized iron hood surrounding reflectors; *E*, air removal pipes connected with hoods and suction fan; *F*, water bath; *G*, perforated water inlet pipe; *H*, constant level device; *I*, drain. Water pump and tin plate reflectors on sides and ends not shown.

temperature of the bath. As the rate of admission is constant for considerable periods of time, the temperature goes down at night and rises during the day, and thermostatic control of temperature would be preferable for many kinds of investigation. In the summer the incoming jets of water are strong enough to cause mixing and prevent thermal stratification in the tank. In the winter, owing to the low temperature of the tap water, the amount allowed to enter is too small to produce jets; and mixing is produced by a circulating pump

(not shown in the figures) from which the water is reintroduced as two jets near opposite corners of the tank.

The light is produced by ten 500 watt gas filled tungsten filament



FIGS. 2, 3.—Fig. 2 (above), side view of plant culture apparatus, with the nearer tin plate reflector removed to show growing plants in place; fig. 3 (below), apparatus for growing a number of plant cultures at one time by artificial light under biologically controlled conditions.

lamps and two 450 watt mercury vapor lamps arranged as shown in figure 1. They are adjustable in height to approximately 1.1 m. (43 in.) above the surface of the water; a greater height would be an

advantage at times. The incandescent lamps can be tilted through a limited angle for equalizing the light over the table. All lamps have white enameled reflectors; additional vertical tin plate reflectors (not shown in figure 1) at the ends and sides of the apparatus extend downward to within about 27 cm. (10.5 in.) of the top of the water tank. The side reflectors are removable for allowing access to the cultures. A time clock, operating through a suitable relay, turns the lights on and off. Any desired relative length of "day" and "night" with a total 24-hour period can be secured.

The light intensity attained a few inches above the surface of the water is about 2200 foot candles[1] (23,700 lux), but varying with the age of the light bulbs in use, the voltage in the line, and the height of the lamps above the water. If this be compared with daylight in the open it may at first seem rather low, since in the latitude of Washington this latter value at noon occasionally exceeds 10,000 foot candles (107,600 lux), and has been known to reach 14,000 foot candles (150,600 lux). The mean intensity, however, during the daily period of illumination for June at Washington, D.C., as an average of 18 years is only approximately 3600 foot candles (39,000 lux). The corresponding figure for December is 2100 foot candles[2] (22,600 lux). It is thus seen that the intensity secured in the apparatus is of a magnitude comparable with out-of-door intensities in which good growth occurs when other factors are favorable. Experience indicates, however, that a greater intensity would often be more favorable, and that certainly it cannot be reduced greatly, for instance by as much as half, without harmful results on the plants.

The spectral quality of the light is of course different from that of sunlight, since so much more of the emission from filament lamps is in the infra-red than is the case with sunlight and since the emission of mercury vapor lamps is in narrow bands. In view of the fact that JOHNSTON (13) has found an excess of infra-red radiation to be injurious to some plants under certain conditions, it might be expected that injuries would occur in this case. No serious bad effects

[1] Intensity measured by Weston photronic cell and by Sharpe-Millar photometer.

[2] Information from the records of the U.S. Weather Bureau. Furnished by Mr. I. F. HAND.

have been noticed which seem to be attributable to this source, however, although the internodes are often longer than normal, especially while the plants are still very young. Possibly the enrichment of the blue and near ultraviolet has prevented real injury in this case. The importance of the shorter wave length portion of the light is further suggested by the results of DASTUR and MEHTA (**7**), who have reported that a proper balance of the spectral elements is necessary for maximum photosynthesis.

The water bath prevents excessive temperatures from developing around the plant roots. The dissipation of the considerable amount of heat produced by incandescent lamps has been one of the greatest problems connected with their use for growing plants (**2, 5, 8, 17, 18, 19**); in at least one recent case it has been found sufficient for heating a well insulated small greenhouse (**3**). One of the points carefully considered in the original design of the apparatus was therefore that of adequate removal of excess heat, in spite of the fact that some workers had already shown that plants can sometimes be grown in artificial light without special measures for heat dissipation (**9, 10, 11**). A heat dissipation device was tried which was composed of a heat absorbing glass filter, cooled by a current of air drawn over it by a suction fan. This filter reduced the intensity of the light too much for economical operation and did not remove the heat as efficiently as was hoped. Experiments soon showed that most ordinary plants can stand without damage the highest air temperatures which are reached in the apparatus without any light filters at all, provided the roots are kept cool. The adoption of the water bath and rejection of the filters accordingly followed. Air temperatures now usually approximate 30° C., and except near the lamps have not been observed above 37°, which is often surpassed in the open. The plants seldom show heat injury until they grow to within about 6 inches (15 cm.) or less from the naked bulb. The air drainage system has been retained as a useful but unessential aid in temperature control. Recently it became desirable to use a much higher light intensity for one experiment. This was attained by using 1000 watt instead of 500 watt lamps and lowering them closer to the plants. Under these conditions the plants showed heat injury. The tin plate sides were then removed and a current of air from a fan was

blown across the table during the remainder of the experiment. No further heat injury occurred.

The importance of keeping the plant roots cool should be emphasized. When the apparatus was first constructed and before the water bath was added, many plants, especially those which can be classed as cool weather plants, did not thrive. A trial with part of the jars standing in a shallow tray of water showed a considerable improvement. The tank was then installed, in which the water is maintained at a depth as great as that of the sand in the jars, or slightly greater, and the improvement was still greater. The tap water in Washington sometimes reaches a temperature as low as 5° C. in the winter, so that it is then possible to keep the roots as cool as any ordinary plant requires. In the summer the tap water exceeds 25° C. much of the time, however, so that it is then impossible to cool the sand below this figure. As best results seem to be secured by a temperature of 20° to 25°, a cooling device for the water in the summer would be an advantage.

During the earlier part of the work attention was concentrated on the growing of subtropical plants, since it was supposed that the high air temperatures would cause plants typical of higher latitudes to fail. Recent experience has shown, however, that alfalfa, sweet clover, sweet peas, garden peas, and other such plants will do well at the temperatures concerned. In fact, in one experiment alfalfa, sweet clover, and sweet peas grew better in the apparatus than they did in sunlight, where, however, the sand became very warm. Cowpeas, on the contrary, did better in the sunlight. It is now believed that most of the ordinary spring or fall (cool weather) crops can be grown satisfactorily. From some standpoints they may be even better suited to the conditions produced in the apparatus than are plants of lower latitudes.

Alfalfa, soybean, garden pea, garden bean, ragweed (*Ambrosia artemisiifolia* L.), pigweed (*Amaranthus retroflexus* L.), partridge pea (*Cassia chamaecrista* L.), and other plants blossomed and produced seed. Sweet pea, sweet clover (*Melilotus alba* Desr.), white clover, alsike clover, red clover, pokeberry (*Phytolacca decandra* L.), golden rod (*Solidago* sp.), millet, Sudan grass, and other plants blossomed but either did not mature seed or data concerning the point were not

secured. Cowpea, lespedeza (*Lespedeza sericea* L. and *L. stipulacea* Maxim.), *Crotalaria spectabilis*, vetch (*Vicia villosa* Roth), and timothy grew well but under the conditions involved did not bloom. An idea of the growth of the plants can be obtained from figure 2, where can be seen alfalfa, sweet pea, soybean, garden bean, cowpea, and Korean lespedeza plants.

The behavior of the plants was very nearly normal except, as already mentioned, for a somewhat greater internode length, especially in the seedling stage, and, as in cowpea, for a slightly variant morphology in other respects. HIORTH (**12**) has reported a similar lengthening of the internodes but he appears to have worked with a lower light intensity. In the present work the addition of the mercury arc radiation was found to improve the plants in this respect. It is therefore likely that the undue lengthening still noted is due at least in part to a lack of balance between long wave and short wave radiation. Further improvement in plant growth could doubtless be obtained by increasing the total light intensity or, likely, by increasing the blueviolet-ultraviolet portion.

There are no actual figures on the operating costs of the apparatus except such as can be figured from the capacity of the lamps. Ten 500watt lamps and two 450watt lamps will use approximately 5.9 kw. per hour. At 2.5 cents per kw. hour, which is the approximate price paid for electric current at this laboratory, the cost is 14.75 cents per hour or $1.76 for a 12-hour day. A total of $2.00 per day as an estimate to cover all operating costs except repairs and replacement of lamps would probably be more than ample at the given kw. hour rate. If it should become practical to substitute glow lamps for the incandescent ones in installations of this kind, as may happen before many years (**4**), the change would reduce the cost of current very materially, as the efficiency of this type of lamp is much greater than that of incandescent lamps.

Apparatus no. 2

This apparatus is an adaptation of a growth chamber first used at the Smithsonian Institution (**6**, p. 128, and pl. 1, fig. 1) in studies of photosynthesis by wheat. The unit for a single culture as used in this laboratory consists of a double walled, cylindrical culture cham-

ber of glass mounted in a wide-mouthed Erlenmeyer flask by means of a rubber stopper with a large hole in it. A tube to the inner chamber at the base serves to introduce air, which may be modified in any way desired. A glass tube through a hole in a rubber stopper at the top permits the air to escape. The space between the inner and outer walls contains flowing water, which serves to keep the interior cool and to filter out much of the infra-red portion of the radiation. Only small leaved plants can be grown with this equipment, as there are mechanical limits to the size of apparatus that can be made at reasonable cost.

In practice, the apparatus, with the flask containing the moist sand and its culture salts, is sterilized in the autoclave. The seeds are sterilized and germinated outside; they are then planted with aseptic procedure, covered with a little sterile sand, and aerated with sterile, moist air throughout the growth period. It is not necessary to add water at any time, as the chamber acts like a reflux condenser. For ordinary plant culture it is sufficient to circulate tap water; for temperature studies it would of course be necessary to have thermostatic control.

It has been found convenient to surround these cylindrical chambers with a housing of tin plate in order to conserve the light, leaving the flasks with the sand below the housing. Figure 3 shows such a housing in use. It would improve the design to put the lights lower so as to increase the light intensity available from a given consumption of electricity.

In this chamber vetch and wheat grow excellently. The only other plant tried, one of the lespedezas, did not do so well. The cause of its failure may have been unsuitable culture salts or wrong temperature. There is thus far no reason to think that the cause was in the apparatus itself.

Summary

1. Two types of equipment used in symbiotic nitrogen fixation and related studies are described. One of these consists of a shallow water bath, an overhead bank of lights, and the necessary accessory equipment for operation. The main features in which it differs from installations previously described are in discarding light filters and air conditioning devices for control of spectral balance and air tem-

peratures, and in substituting mercury vapor lamps and a water bath. It was found that most plants will grow well at the highest air temperatures reached if the spectral balance is fairly good and the roots do not become too warm. The intensity of illumination secured is usually about 1500 to 2200 foot candles (16,100 to 21,500 lux). With 1000 watt lamps over 4000 foot candles (43,000 lux) has been attained. Most plants grow in a practically normal manner in the apparatus.

2. The second apparatus consists of a unit composed of a water jacketed glass culture chamber mounted by means of a rubber stopper in a wide mouthed Erlenmeyer flask which contains the material in which the roots grow. A number of such units are assembled in a tin plate housing to conserve the light. Good growth of small leaved plants under biologically controlled conditions is easy to secure with the apparatus.

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EXPERIMENTS CONCERNING DIFFUSION OF NITROGENOUS COMPOUNDS FROM HEALTHY LEGUME NODULES OR ROOTS

C. A. LUDWIG AND FRANKLIN E. ALLISON

(WITH TWO FIGURES)

Introduction

It has long been common knowledge that the cultivation of legumes followed by their addition to the soil increases soil nitrogen. Occasionally also it has been claimed that the soil can be enriched in nitrogen before the death of the legumes and even while they are young and growing vigorously. Most of the recorded experiments on this point have been inconclusive, although sometimes very favorable to the idea. Recently, however, VIRTANEN and his associates (**11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22**), of Helsingfors, Finland, have carried out a series of rigidly controlled experiments in which they found, not only that nitrogenous compounds are given off into nitrogen-free sand in this way but that enough of such compounds are given off to permit normal development of non-legumes growing in the same sand. This whole subject has recently been reviewed in detail by NICOL (**7**). The present work is along the same line and represents chiefly an attempt to duplicate their results, mostly with other crop plants and under somewhat different conditions. A preliminary statement was presented at the New York meeting of the Society of American Bacteriologists in December, 1935, and an abstract has been published in connection with that meeting. (**6**).

Methods

Most of the cultures have been grown with artificial light in an apparatus described in this issue (**5**) which has been found suitable for the growth of many plants. In this apparatus the source of the light is ten 500 watt Mazda incandescent lamps and two 450 watt mercury vapor lamps in glass, which together give an intensity of about 2200 foot candles (23,700 lux) at the surface of the sand in

which the plants grow. Some other cultures were grown in a modified coldframe in the open. Culture vessels have been for the most part approximately 4-liter glazed earthenware jars containing 4.5 kilos of an almost nitrogen free washed quartz sand to which minerals except nitrogen were added, either in solution or as a coarse powder.

Several mineral mixtures were tried but most of the work was done with one of the following three:

1. A fertilizer mixture used by GIÖBEL (**2**) for growing alfalfa, and modifications of the same. This proved unsuitable, after thorough trial, for the growth of most plants, and has been discarded except for a special case mentioned later.

2. A solution used by VIRTANEN and associates (**17**, p. 63) for growing legumes. This contains:

$MgSO_4 \cdot 7H_2O$	1.2 gm.	KH_2PO_4	1.4 gm.
KCl	1.5	Water	20 liters

As used by VIRTANEN it contains a trace of ferric chloride also, but as used here this was omitted and instead 0.6 mg. per culture of iron combined with humic acid extracted from soil was supplied at planting time. One third gram of tricalcium phosphate per kilogram of sand is mixed dry with the sand previous to planting and the solution is used to water the plants.

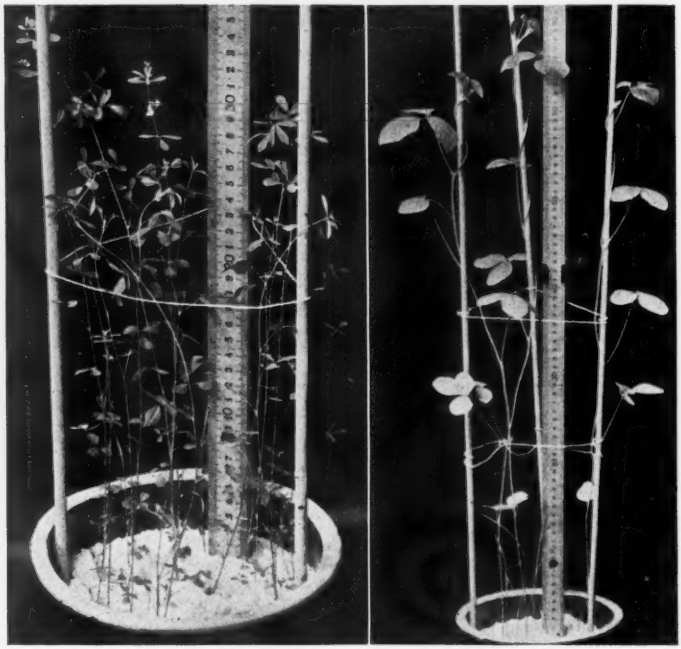
3. A solution used by THORNTON (**8**) and associates, also for growing legumes. As used here it contains:

KCl	0.74 gm.	NaCl	0.5 gm.
K_2HPO_4	0.3	$CaSO_4$	0.5
KH_2PO_4	0.3	Water	1 liter
$MgSO_4 \cdot 7H_2O$	0.5		

Iron was added at the rate of 0.6 mg. per culture in combination with humic acid extracted from soil. The solution is used to moisten the sand at the beginning of an experiment and more is added later if the growth of the plants seems to make it necessary. This solution proved to be the most successful of any tried thus far.

In most cases 1 mg. per culture of boron as sodium borate was also added. It was sometimes necessary to repeat the addition of boron during the course of the growth period in order to prevent death of the growing buds.

Cultures grown in this way are not sterile, although in some cases an initial sterilization of sand and seeds was made. Sterilization is probably of small importance, since VIRTANEN obtained his results with both sterile and non-sterile cultures. Common contaminants



FIGS. 1, 2.—Fig. 1 (left), alfalfa, Hungarian millet, and timothy culture 35 days after planting the alfalfa. Fig. 2 (right), soybean, Sudan grass, and Hungarian millet culture 33 days after planting the soybeans. In both cases the grasses were planted after the legumes were well started. Note bloom on one alfalfa plant and small size of the grasses in each culture.

in the present cultures were one or more species of algae, some of which appear to have been nitrogen fixing forms; but the results indicate that the amount of nitrogen fixed was never enough to cast doubt on the conclusions. However, some cultures which were sterile as to organisms other than the nodule bacteria have been carried out. These were conducted in double walled, water cooled growth cham-

bers similar to that used at the Smithsonian Institution (1, 3, 5) for studies in photosynthesis. Only small leaved plants can be grown in these chambers; vetch and wheat were used in this work. The chambers were mounted in wide mouthed Erlenmeyer flasks each containing about 4 kg. of sand. The sand contained 1.25 gm. per kilogram of a modified GIÖBEL's fertilizer mixture. This contains the following substances, finely ground together:

Ground marble.....	4 parts	KCl.....	5 parts
Superphosphate.....20		$MgSO_4 . 7H_2O$.....	2

Iron was added separately as iron humate at the rate of 0.3 mg. Fe per culture. The apparatus was sterilized for one to two hours in the autoclave at 18–19 pounds' pressure. The air which was passed through the chambers was enriched to about 0.5 per cent with CO_2 to improve photosynthesis, and was bubbled through strong sulphuric acid and a mercuric chloride solution to remove any combined nitrogen, to sterilize it, and to humidify it. Seeds were sterilized by treating for 5 minutes or more with concentrated H_2SO_4, rinsing a few times in tap water, immersing in 1/1000 $HgCl_2$ solution for 5 minutes or a little more, and rinsing six times or more with sterile water.

The moisture content usually desired for the cultures was about 12 per cent. If more water is added it flows to the bottom of the jar or flask and does not increase appreciably the moisture content of the bulk of the sand. It should be noted, however, that much of the time the moisture content varies greatly in the cultures open to the air, since as soon as the plants attain a considerable size they transpire water so rapidly that a jar often loses in one day almost or quite as much water as is necessary to make a moisture content of 12 per cent. It was not often practical to water more than once a day, and of course after plants reached this stage it was very impractical to attempt to regulate moisture closely by bringing the jars to weight occasionally, although this was done while the plants were small. With the sterile cultures it was not necessary to add water at any time, as the growth chamber acted as a reflux condenser and returned the transpired water to the sand.

The following legume plants have been used at one time or an-

other: cowpea (*Vigna sinensis* (L.) Endl.), soybean (*Soja max* (L.) Piper), alfalfa (*Medicago sativa* L.), vetch (*Vicia villosa* Roth), sweet pea (*Lathyrus odoratus* L.), sweet clover (*Melilotus alba* Desr.), red clover (*Trifolium pratense* L.), pea (*Pisum sativum* L.), bean (*Phaseolus vulgaris* L.), and Korean lespedeza (*Lespedeza stipulacea* Maxim.). The following non-legumes have been used: Sudan grass (*Sorghum vulgare sudanense* (Piper) Hitchc.), millet (species uncertain), Hungarian millet (*Setaria italica* (L.) Beauv.), pigweed (*Amaranthus retroflexus* L.), wheat (*Triticum aestivum* L.), timothy (*Phleum pratense* L.), oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.), and Italian rye grass (*Lolium multiflorum* Lam.).

At the close of the growth period the plants were harvested and separated into legume tops, non-legume tops, and (where possible) legume roots and non-legume roots. In many cases, however, it was impractical to separate the roots of the two kinds of plants and they were analyzed together. The airdry weight of the tops was usually taken but that of the roots was omitted on account of the clinging sand. These weights are omitted in the following discussion, however, as they parallel fairly closely the nitrogen found. Sand extracts were made in the earlier experiments by putting the sand in a glass cylinder and allowing cold water to percolate through. In the later extractions (experiments VI, VII, and VIII) a special apparatus was used in which an initial cold water extraction was followed by intermittent extraction for several hours with water at nearly the boiling point. Nitrogen determinations were by the Kjeldahl method.

Results

The results, except as regards total nitrogen in the sand, are given in table 1. It will be observed that, with one exception, whether the criterion adopted be the nitrogen accumulated by the non-legumes or the water extractable nitrogen in the sand, the indications are the same throughout the entire series of experiments; namely, that under the conditions concerned little or no nitrogenous material was excreted from legume nodules or roots into the substratum. In fact, the non-legumes grown in association with legumes were usually smaller and accumulated less nitrogen than the very small ones grown alone. The amount of water extractable nitrogen in the sand

TABLE 1

NITROGEN ACCUMULATION BY NON-LEGUMES GROWING IN ASSOCIATION WITH LEGUMES IN DIFFERENT EXTERNAL CONDITIONS

EXP. NO.	NO. OF CULTURES	SPECIES AND APPROXIMATE NO. OF PLANTS USED		MINERAL MIXTURE OR SOLUTION USED	APPROXIMATE DURATION OF EXPERIMENT (MONTHS)	NITROGEN ADDED IN SEEDS		N_2 FIXED[a] (MG.)	N IN NON-LEGUMES LESS THAT IN SEEDS PLANTED			WATER[a] SOLUBLE N IN SAND AT CLOSE (MG.)	REMARKS
		LEGUME	NON-LEGUME			LEGUME (MG.)	NON-LEGUME (MG.)		CONTROL (MG.)	EXPERIMENTAL[a] (MG.)	GAIN OR LOSS BY ASSOCIATION[a] (MG.)		
II	2	1 cowpea[b]	pigweed, Sudan[c] grass, millet	Gïobel[d]	8	5.7	1.0	335–433	.....	0.5 – 1.1[e]	.....	1.2– 2.6	.....
III	1	2 cowpea	1 pigweed, 1 Sudan grass, 1 millet	Baked soil	3.7	11.06	0.3	43	0.54[f]	0.05[f]	−0.49	.....	.....
	1	2 cowpea	" "	Gïobel[d]	3.7	11.86	0.3	51	1.13[f]	0.07[f]	−1.06	0.6	.....
	1	2 cowpea, uninoculated	" "	"	3.5	11.86	0.3	3	1.13[f]	0.54[f]	−0.59	0.4	.....
	3	2 cowpea	" "	Hopkins[d]	3.3	10.39	0.3	30–66	0.21[f]	0.03– 0.16[f]	−0.18 to −0.05	0.6– 1.6	.....
	1	2 cowpea, uninoculated	" "	"	3.3	10.39	0.3	3	0.21[f]	0.08[f]	−0.13	1.0	.....
IV	2	cowpea	2 pigweed, 1 Sudan grass, 1 millet	Modified[d] Gïobel	5.5	.....	.....	82–96	2.8	[g]	.....	.....	.....

[a] Where more than one value is concerned the two extremes are given rather than the mean.

[b] One culture grown in natural light for a time.

[c] Planted at beginning of seventh month, after fixation was well established.

[d] Gïobel's fertilizer mixture, as used in experiments II and III, gave a slightly alkaline reaction, usually pH 7.0 to 8.0 but more often nearer 8.0 than 7.0. As modified and used in experiments IV, V, VI, and VII its reaction was about neutral. The Hopkins solution gave a reaction usually about pH 7.3 to 7.8; Thornton solution in sand gave a reaction of about neutrality.

[e] Tops only.

[f] Tops only, not corrected for nitrogen added in seed.

[g] Through an error, the nitrogen in the legume and in the non-legume plants was not determined separately. However, the mean airdry weight of the tops of the non-legumes grown in association with cowpeas was 0.111 gm. while corresponding weight of plants grown alone was 0.302 gm.; evidently no significant absorption of nitrogen from the legumes can have taken place.

TABLE 1—*Continued*

Exp. no.	No. of cultures	Species and approximate no. of plants used		Mineral mixture or solution used	Approximate duration of experiment (months)	Nitrogen added in seeds		N_2 fixed[a] (mg.)	N in non-legumes less that in seeds planted			Water[a] soluble N in sand at close (mg.)	Remarks
		Legume	Non-legume			Legume (mg.)	Non-legume (mg.)		Control (mg.)	Experimental[a] (mg.)	Gain or loss by association[a] (mg.)		
V[h]	2	2 vetch	2 wheat	Gïobel[d]	2.3–3.5	2.88	1.8	67–193	.....	1.3–11.1	.....	9.5–10.4	.....
VI[b,i]	9	2 vetch	2 wheat	Gïobel[d]	3–3.5	1.41	1.88	46–114	0.7	1.8	1.1	5.3– 8.8[k]	.....
	1	2 vetch, uninoculated	2 wheat	Gïobel[d]	.....	1.41	1.88	11	0.7	0.4	−0.2[j]	9.1[k]	.....
VII[h]	2	2 vetch	2 wheat	Gïobel[d]	2.5	1.30	1.88	153	.....	2.0– 2.3	.....	6.0– 6.1[l]	.....
VIII	8	9 alfalfa	4 Sudan grass	Thornton[d]	4.5–5	.....	.....	55–301	.....	1.2– 3.3[f]	.....	9.9–17.1[m]	.....
IX	5	15 alfalfa	8 timothy, 4 Hungarian millet, and 4 Sudan grass	Thornton[d] and Virtanen	4	3.09	1.10	160–380	3.5	0.0– 2.1	−3.5 to −1.4	.....	.....
	5	15 alfalfa	" "	"	4	3.09	1.10	280–387	3.5	0.0– 0.4	−3.5 to −3.1	.....	Aerated
	4[n]	15 alfalfa	8 timothy and 4 Hungarian millet	"	3.7	3.09	0.31	43–98	5.0	0.8– 3.0	−4.2 to −2.0	.....	Sunlight
	2	15 sweet clover	8 timothy, 4 Hungarian millet, and 4 Sudan grass	"	4	2.83	1.10	173–204	3.5	1.0– 1.8	−2.4 to −1.7	.....	.....
	2[n]	15 sweet clover	8 timothy and 4 Hungarian millet	"	2.2	2.83	0.31	70[o]	5.0	0.0– 1.5	−5.0 to −3.5	.....	Sunlight
	5[n]	6 vetch	5 Hungarian millet and 1 oat	"	2	14.80	2.08	12–75	0.0	0.6– 1.1	−0.6 to −4.9	.....	Sunlight
	6	4 sweet pea	2 Hungarian millet and 2 Sudan grass	"	3.3	21.02	0.99	83–125	4.8	2.5– 4.9	−2.4 to −0.1	.....	.....
	6	4 sweet pea	" "	"	3.3	21.02	0.99	38–90	4.8	1.7– 5.6	−3.1 to 0.8	.....	Aerated
	5[n]	4 sweet pea	" "	"	2	21.02	0.99	8–16	3.2	0.7– 4.2	−2.6 to 0.9	.....	Sunlight
	2	3 cowpea	2 Hungarian millet and 4 Sudan grass	"	3.7	24.71	1.39	436–514	4.8	0.1– 0.2	−4.7 to −4.6	.....	.....
	2[n]	3 cowpea	" "	"	3.7	24.71	0.60	901–906	3.2	0.2– 1.0	−2.5 to −2.2	.....	Sunlight

[h] Cultures sterile except for presence of nodule bacteria.

[i] Light intensity for these cultures only a little more than half as great as for nos. V and VII.

[j] Apparent error in subtraction is due to carrying out the calculations to further digits and rounding off the final results.

[k] Extraction started with cold and finished with hot water. The following additional values can be given: one culture, inoculated vetch only, 6.8 mg.; two cultures, uninoculated vetch only, 8.4–8.8 mg.; four cultures, wheat only, 6.9–9.1 mg.; two flasks, no plants, 5.5–5.7 mg. The variation in these results apparently does not exceed experimental error.

[l] Extraction started with cold and finished with hot water.

[m] Extraction with cold and hot water. Sand from three cultures only, extracted. Extraction of sand from grass-only culture secured 8.2 mg. nitrogen, and from alfalfa-only culture, 12.1 mg. nitrogen.

[n] Cultures grown in modified coldframe.

[o] Mean of the two cultures, accidentally put together for analysis.

TABLE 1—*Continued*

Exp. no.	No. of cultures	Species and approximate no. of plants used		Mineral mixture or solution used	Approximate duration of experiment (months)	Nitrogen added in seeds		N_2 fixed[a] (mg.)	N in non-legumes less that in seeds planted			Water[a] soluble N in sand at close (mg.)	Remarks
		Legume	Non-legume			Legume (mg.)	Non-legume (mg.)		Control (mg.)	Experimental[a] (mg.)	Gain or loss by association[a] (mg.)		
X	4	10–18 alfalfa	6 timothy and 3 Hungarian millet	Thorton[d]	3	3.09	3.89	393–516	2.1	0.5 –1.3	−1.6 to −0.8	.....	Usual light[p]
	4	10–18 alfalfa	" "	"	3	3.09	3.04	291–348	2.1	0.8 –1.1	−1.4 to −1.0	.....	Low light[p]
	1	9 sweet clover	" "	"	3	2.96	6.85	864	2.1	2.2	0.1	.....	Usual light
	1	9 sweet clover	" "	"	3	2.96	5.99	372	2.1	1.8	−0.2	.....	Low light
	1	14 red clover	" "	"	3.3	2.16	6.05	21	2.1	3.2	1.1	.....	Usual light
	4	4 sweet pea	3 Hungarian millet and 3 Sudan grass	"	3.2	15.76	4.63	308–430	2.5	2.2 –8.1	−0.3 to 5.6	.....	Usual light
	4	4 sweet pea	" "	"	3.2	15.76	4.41	193–279	2.5	0.8 –2.9	−1.7 to 0.4	.....	Low light
	1	4 pea (Nott's Excelsior)	" "	"	2.7	55.16	4.63	303	2.5	2.15	−0.4	.....	Usual light
	1	3 garden bean	" "	"	3.0	54.74	4.63	85–150	2.5	0.7 –7.0	−1.8 to 4.5	.....	Usual light
	2	3 cowpea	" "	"	2.8	30.00	4.63	100–114	2.5	0.4 –0.7	−1.8 to −2.1	.....	Usual light
	1	3 soybean	" "	"	2.8	39.23	4.63	453	2.5	0.9	−1.6	.....	Usual light
	1	3 soybean	" "	"	2.8	39.23	4.41	401	2.5	0.1	−2.4	.....	Low light
	1	16 Korean lespedeza	6 timothy and 3 Hungarian millet	"	3.0	2.84	3.89	249	2.1	2.7	0.6	.....	Usual light
XI	12	4 sweet pea	1 barley, 1 oat, 3 timothy, and 3 Italian rye grass	"	2.2	.....	.....	211 or more	.....	.....	1.7	.....	Usual light
	2	4 pea (Nott's Excelsior)	" "	"	2.1	.....	.....	223–227	.....	.....	1.4	.....	Usual light
	2	3 pea (Torstai)	" "	"	2.1	.....	.....	181–275	.....	.....	2.5	.....	Usual light
	2	4 Canada field pea	" "	"	2.1	.....	.....	149–219	.....	.....	−0.2	.....	Usual light
	2	4 hairy vetch	" "	"	2.1	.....	.....	261–296	.....	.....	1.2	.....	Usual light

[p] Usual light, about 2200 foot candles or 23,700 lux; low light, about 1500 foot candles or 16,100 lux; high light, about 4200 foot candles or 54,200 lux (obtained by using 1000 instead of 500 watt lamps).

at the close of those experiments where it was determined was not significantly different between experimental cultures and controls and in some cases was also negligible in amount.

In experiments II and III determinations of total nitrogen in the sand were also carried out. The results were highly variable and reflected the difficulty of making such determinations rather than any differences due to the treatments. They are therefore not included in the table.

Discussion

It should perhaps be stated at the outset that it is not believed that these results show that VIRTANEN and others who have secured apparently opposing results are in error. It is considered that neither set of results is to be so interpreted but that some difference in conditions has caused the contrast. To date it has not been possible to determine this difference, but the results do furnish evidence concerning certain possibilities, some of which are discussed next.

GOOD GROWTH *vs.* POOR GROWTH.—In the earlier experiments the growth and nitrogen fixation by the legumes were not very satisfactory. This is the case for experiments II, III, and IV, and especially so for no. I, which is not included in the table for that reason. In the later experiments excellent growth was obtained in many cases, as well as poor growth in others. Figures 1 and 2 show an alfalfa and a soybean culture at age 35 and 33 days, respectively, and show the relative growth of legume and non-legume in the better mixed cultures. VIRTANEN'S results have usually been secured with cultures where growth was rapid; but in at least one case (**17**, p. 62), where the growth rate was reduced by altering the reaction of the sand, practically the same proportional excretion was obtained with the slowly growing plants as with the others.

FLUCTUATION OF CONDITIONS OF ENVIRONMENT OR OF PLANTS.—It is conceivable, although unlikely from the reports of those observing the phenomenon, that certain fluctuations in environment or internal activity of the legumes may be necessary or favorable to the excretion observed. In the work considered here several such cases of fluctuation have occurred, sometimes by design and sometimes in spite of all efforts at prevention. Among them may be mentioned the following.

(1) In experiment X the cultures were grown at the usual light intensity until it seemed evident that the result was negative and would continue so. At that time some of the cultures of alfalfa, sweet pea, sweet clover, and soybean were transferred to a compartment with about three-fourths the original light intensity. The transferred plants must have undergone an alteration in metabolic activity following the transfer. In addition, at the time of transfer, two alfalfa and two sweet pea cultures were left in practical darkness for 3 days, one culture of each being then returned to each light condition. This treatment was drastic enough to cause detectable etiolation of the portions of the stems which elongated during the period and to cause death of the developing blossom buds on the alfalfa plants. It can hardly be doubted that there was a considerable interference in the metabolism of these plants, involving a reduction in the C/N ratio.

(2) The alfalfa, sweet clover, and vetch cultures in experiment IX were clipped once or twice before the final harvest was made. The sudden reduction in photosynthesizing tissue would be expected to alter temporarily the metabolic conditions in the roots, again in the direction of a lower C/N ratio.

(3) In experiment VIII the alfalfa grew well for a time, after which a period of severe decline set in, caused principally by thrips infestation. The insects were poisoned, after which a second period of good growth occurred.

(4) In experiment II the cultures were continued until the cowpea plants were deteriorating almost to the point where root disintegration had begun. Likewise in experiment IX some of the sweet pea plants in artificial light and all of the legumes in the outdoor cultures were deteriorating at the close.

(5) In all cultures except the sterile ones the amount of moisture in the sand fluctuated, in some cases enormously.

RELATION OF SPECIES TO RESULTS.—Suggestion has been made that the use of the same strains of green plants and bacteria used by VIRTANEN and his associates might have given the same results which they obtained. Such a possibility always exists, of course, so long as a thorough trial is not actually made. It seems unlikely, however, since (a) alfalfa, which is one of the plants employed here, has

been reported by THORNTON and NICOL (**7, 9, 10**) to give the result; (b) WILSON (**23**) has reported tests in this country in which VIRTANEN'S organisms were used but with negative results; (c) mixed cultures of garden pea and grasses reported here, including in a few cases the varieties used by VIRTANEN, did not indicate that the grasses were improved by being grown in association with the pea plants; and (d) VIRTANEN has evidence (**15**) to indicate that alders (*Alnus* sp.) give the result as well as peas and clover, thus indicating that the phenomenon is fairly general so far as species is concerned. It is true, of course, that all workers who have reported positive results, from LIPMAN (**4**) to the present, have used what may be termed cool weather plants. Possibly this is due merely to the coincidence that these workers lived in a cool temperate zone, but LIPMAN did not get good results with cowpeas and soybeans, and the possibility of such a climatic correlation remains. Incidentally it may be remarked that winter wheat sowed shortly after harvesting a crop of soybeans is usually reduced in yield, owing chiefly to the removal of most of the available nitrogen from the soil by the soybeans.

INTENSITY, SPECTRAL BALANCE, AND DAILY DURATION OF LIGHT.—The experiments involved several different light intensities and durations per day. Thus the plants in experiment IV were grown for a considerable time under 12 hours of daily illumination but for the last 25 days were under 20 hours. The plants in experiment VI had about half the light intensity of those in nos. V and VII. One culture in experiment VIII was started in sunlight but was soon transferred to artificial light and 12 hours of illumination per day. The artificial light cultures of experiment IX were illuminated 12 hours per day, while those in sunlight had the illumination periods, light intensities, and spectral balance occurring naturally from April 24 to August 21. In experiment X artificial light of 18 hours' duration per day was used in two intensities. In experiment XI the duration was 18 hours per day and the intensity about twice that of the higher intensity in no. X. The one culture in experiment V which gave a possible positive result was in one of the higher light intensities, and might be considered to indicate that high light intensity will cause the excretion of nitrogen except for the fact that a com-

panion culture in the same experiment and two cultures in the same intensity in experiment VII gave negative results.

TEMPERATURE.—No study, as such, has been made of the effect of different temperatures or of fluctuations in temperature, but there have been seasonal as well as diurnal fluctuations in temperature, both of air and of sand, during the course of the work. Some experiments have been conducted at lower temperatures than others, and none has been at a fixed temperature. Except in the sterile cultures, air temperatures as a rule have been rather high, but not exceeding that of a bright summer day.

REACTION OF SAND.—No specific investigation has been made of the effect of the reaction of the sand. However, the earliest cultures were carried out at slight alkalinity and later ones at about neutrality. Further work at somewhat lower pH values is to be desired.

AERATION.—Recently VIRTANEN and his collaborators have reported (**18, 20, 21**) that increased aeration appears to increase the amount of nitrogen excreted. Still more recently he has reported (**14**) that the effect seems to be an indirect one, due to an increase in nitrogen fixed rather than to the percentage excreted. In order to determine whether additional aeration would cause the initiation of nitrogen excretion under the conditions of these studies, part of the cultures in experiment IX were subjected to extra aeration of the sand. A 7–8 cm. funnel was inverted in each treated jar in such manner that it reached to within 2 cm. of the bottom of the jar. The jar was then filled with sand, the seeds planted in the usual way, and the stem of the funnel connected with a pressure cylinder containing the air to be used. A wash bottle containing $HgCl_2$ solution was interposed in order to moisten and incidentally to sterilize partially the flowing gas. A slow stream of air enriched to 0.5 per cent with CO_2 was passed through the sand of the treated jars in the early part of the experiment; later ordinary air was used. The growth obtained and the amount of nitrogen fixed (table 1) were about the same whether the sand was aerated or not. A slight advantage for the unaerated cultures may have been due to better light, as it was necessary to place the aerated cultures permanently at one end of the bench under slightly less than average intensity. While the

added aeration did not seem to have any significant effect on the behavior of the plants, it should be remembered that the comparison involved is not between poorly aerated and well aerated cultures but between well aerated and unusually well aerated cultures, and that the results might possibly be different under the former conditions.

MOISTURE OF AIR.—The tops of the plants in most of the cultures were exposed to extremely arid conditions but with those plants grown under controlled bacterial conditions the air was much more moist. The fact that the one result which seems to be positive occurred under the latter circumstances might appear to be suggestive if it were not that the other experiments of the same kind did not produce the same result.

PROPORTION OF NON-LEGUMES TO LEGUMES.—In the beginning of an experiment the proportion of non-legumes to legumes was usually somewhat less than that used by VIRTANEN, owing partly to the use of somewhat smaller numbers of plants and partly to the more frequent use of species with small seedlings. Toward the close of an experiment it was often much less if considered on a basis of tissue mass or root surface, because the non-legumes grew very little after their seed reserves were exhausted. In view of VIRTANEN'S equilibrium hypothesis (14), and particularly of his statement that an increase in the relative amount of non-legumes leads to a greater excretion from the nodules, it seems possible that an increase in the proportion of non-legumes to legumes might have led to positive results. But even if this were true it seems unlikely that any positive results so obtained would have been anywhere nearly as large as those reported by them, since the non-legumes used here showed no significant gains in practically all cases. In order to test their ability to absorb available nitrogen during the latter part of the experiments, approximately 100 mg. of nitrogen as NH_4NO_3 was added to each of two of the alfalfa cultures at about the time of the second clipping in experiment IX. As was to be expected, the grass plants, although very small and unhealthy in appearance, showed in proportion to their size roughly the same ability to assimilate the added nitrogen as did the much more vigorous alfalfa plants. The evidence suggests strongly that if considerable amounts of excreted nitrogen

had been present in the sand the non-legumes would have assimilated enough of it to give results of undoubted significance in most cases.

CHARACTER OF SUBSTRATUM.—A small sample of the sand which VIRTANEN used has been received from him. As it is somewhat finer than that used in these cultures it is barely possible, in view of his statement (**14**) that excretion is increased by a medium of high adsorptive capacity, that the use of a finer sand or one mixed with kaolin or other colloid might have induced the result.

Summary and conclusion

1. Mixed cultures of inoculated legumes and non-legumes have been grown in nearly nitrogen free quartz sand and minerals, and observations made on the growth, on the nitrogen accumulated by the non-legumes, and in some cases on the total and water extractable nitrogen in the sand.

2. Instead of furnishing nitrogen to the non-legumes grown in association with them, the legumes usually reduced further their already very meager growth and small nitrogen assimilation from the medium. In one case, a vetch-wheat culture, a slight benefit was noted but later experiments under similar conditions gave negative results. Likewise, in contrast to VIRTANEN'S results, no excreted soluble nitrogenous matter could be extracted from the sand with water. The failure to duplicate his results does not appear to have been due to unsatisfactory growth of the plants, unchanging environmental conditions, failure to use the right species, improper intensity, spectral balance, or duration of the light, unsuitable temperature, too great acidity of the sand, or insufficient aeration of the sand. Too few data have been secured for a judgment as to the possible effect of lowering the pH to 6.5 or below, of an increase in the humidity of the air, of a considerable increase in the non-legume/legume ratio, or of the use of a medium of considerably higher adsorptive capacity; and it is possible that one of these conditions or a combination of some of them might induce the result.

3. These considerations indicate rather strongly that in the case of a considerable number of legume species, under conditions producing good growth, the heavy excretion of nitrogenous compounds observed by several workers does not always occur.

Thanks are extended to ELLEN K. RIST of this laboratory for making most of the nitrogen determinations.

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PERSISTENT JUVENILES AMONG THE CYCADS

M. A. CHRYSLER

(WITH SIXTEEN FIGURES)

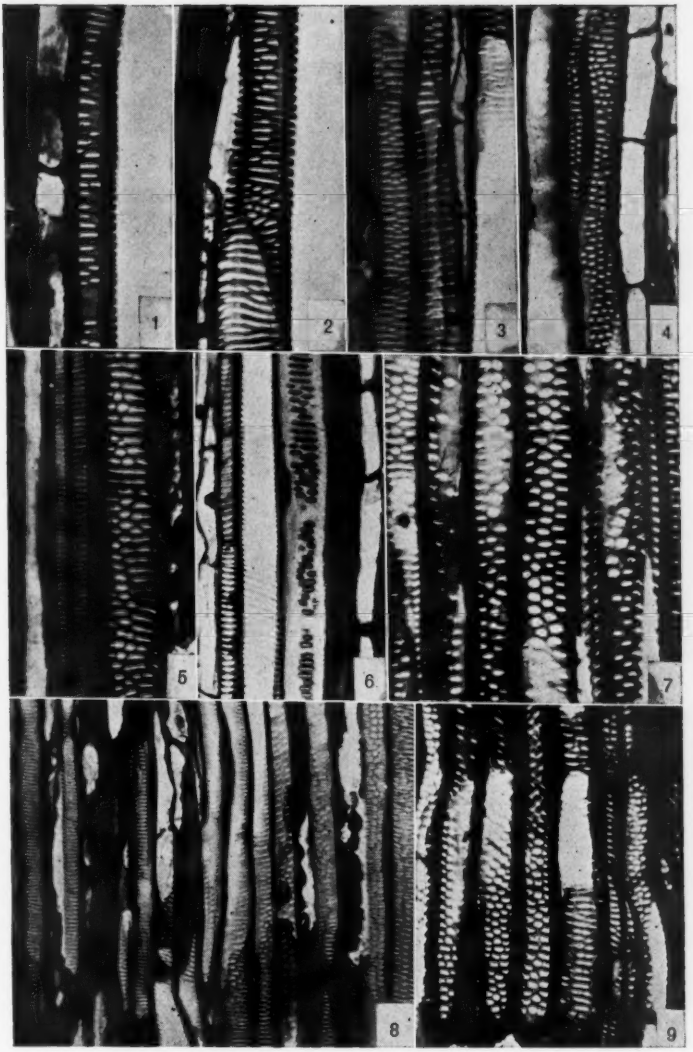
Introduction

It has long been known that the characteristic structural element of the xylem of Cycadales is the pitted tracheid. These elements, together with parenchyma and rays, make up the bulk of the wood in the stem in seven out of the nine genera. *Zamia* and *Stangeria* alone show in their stems a xylem consisting of scalariform tracheids, together with the usual parenchyma. In all genera which have been examined, the tracheids near the pith present scalariform or reticulate markings, and in some genera, for example, *Microcycas* (3), there is a gradual transition from the scalariform to the pitted condition, until finally the latter becomes established.

Suspecting that the reports on the scalariform condition in *Zamia* might be due to examination of relatively immature plants, the writer secured from Florida a plant of *Z. floridana* having a caudex measuring 130 mm. in diameter at its greatest width, and bearing five megasporangiate cones. Sections of this stem showed none but scalariform tracheids, except near the pith, where tight spirals and reticulate thickenings were in evidence.

A little later a study of leaf bundles of this species was undertaken, and BAILEY'S report (1) of transitional and pitted tracheids in the centripetal xylem of the rachis was confirmed (figs. 1–3). Since the leaf is regarded as a conservative organ compared with the stem, the presence of the advanced condition of bordered pits appears surprising if not disconcerting from a morphological standpoint—the leaf ought to be more primitive than the stem, rather than less primitive. Is the doctrine ("canon" is JEFFREY'S 5 word) of conservative organs discredited by these observations?

The answer to this question has been furnished by a study of species of *Zamia* possessing a trunk. All of the species native to Florida and the West Indies have a tuberous stem or caudex, and



FIGS. 1–9.—Fig. 1, *Zamia floridana*, pitted tracheid from centripetal xylem of leaf rachis. Fig. 2, same, leaf rachis, ends of two tracheids showing transitional stages. Fig. 3, same, leaf rachis. Contact between tracheids and parenchyma, showing half-bordered pits. Fig. 4, *Z. kickxii*, leaf rachis; pitted tracheids. Fig. 5, *Z. furfuracea*, leaf rachis; transitional stages. Fig. 6, *Z. pseudoparasitica*, leaf rachis; spiral and pitted tracheids. Fig. 7, same, outer region of xylem of stem showing multiseriate bordered pits. Fig. 8, same, inner region of xylem of stem showing scalariform, transitional, and pitted tracheids. Fig. 9, *Z. tuerckheimii*, outer region of xylem of cone axis showing transitional and pitted tracheids.

anatomical studies have apparently been restricted to these. In Central America, however, there are several representatives of the genus possessing well defined trunks. Through the generous cooperation of Drs. A. SKUTCH and W. R. HATCH, material of trunk-forming species has been collected in Costa Rica and Guatemala. An examination of this material has disclosed entirely normal pitted tracheids in the stem wood (fig. 7), just as occurs in other trunk-forming genera such as *Dioon* (2).

Before proceeding to a description of the new material, it may be pointed out that the occurrence of pitted tracheids in the trunk wood of these species at once explains the occurrence of such elements in the petioles of the various species, including those with a tuberous stem. In this respect the petiole is neither more nor less primitive than the stem, whatever other conservative features the petiole may show. It would appear that the tuberous *Zamia* species have petioles whose xylem shows pits because their ancestors were treelike and had a xylem like that of the other genera of cycads.

Observations

STRUCTURE OF TRUNKS

The material provided by Dr. SKUTCH has been identified as *Z. pseudoparasitica* Yates, collected in heavy forest, Prov. San José, Costa Rica, at an altitude of 1070 m. It is reported to have a trunk up to 1 m. high, with leaves almost 2 m. long. The pieces of stem used in this work are 70 mm. in diameter, and the xylem ring measures 3–4 mm. in thickness. The general features of this layer are entirely typical of cycads such as *Dioon*, consisting of wide tracheids with a sprinkling of parenchyma cells, and rays of varying width. For a thickness of about forty tracheids nearest the phloem, the uniform pattern of thickening is circular bordered pits, as is represented in figure 7. Toward the pith, however, the markings are transitional (with slitlike and partly scalariform markings), and finally scalariform and scalariform-reticulate (fig. 8).

Dr. HATCH'S material includes two species, the first of which has been identified as *Z. skinneri* Warsc. It was found on "limestone outcrops, under a rather dense canopy in montane or lowland rainforest," on Rio Chiacte, Guatemala, altitude 1300 m. The trunk is

described as prostrate and sinuous for 1 m., then erect for 1.2 m., width tapering from 120 mm. at the apex to 50 mm. at the soil line. The block which has been cut measures 75 mm. in width, and has a xylem ring 2 mm. thick. As in the preceding species, the inner region of the xylem shows scalariform tracheids, while the outer region presents a truly remarkable appearance on account of the tertiary rings or spirals which are plentifully laid down upon the pitted walls. As may be seen from figure 13, the pits are circular, bordered, and closely disposed, while the rings are stout and conspicuous. In some cases the rings lie over pits, while in others they lie between pits. Scalariform and reticulate walls as well as pitted ones may be reinforced by these thick rings. Tertiary thickenings on the walls of tracheids in cycads have been mentioned by SIFTON (**8**). In the absence of illustrations, it is difficult to determine whether SIFTON referred to such extremely stout annular thickenings as are so abundant in these trunk-forming *Zamia* species.

The other specimen from Guatemala, *Z. tuerckheimii* Donn. Smith, was collected in the same habitat as *Z. skinneri*, at Alta Verapaz, and had the same habit of growth, being prostrate for nearly a meter, then erect for 0.4 m. The pieces under study have a diameter of 50–90 mm., and the xylem is 1 mm. thick, showing a series of only about twenty-five tracheids, all of which have scalariform thickening. The considerable length of this trunk, the exceedingly thin vascular ring, and the high rainfall of the habitat (average of seven years is reported as 178 inches annually) indicate that this plant grew rapidly and is a comparatively young specimen. It bore a single cone while in the case of *Z. skinneri* cones were found in abundance, the micro-cones in groups of 2–6 on a single plant. If we are correct in regarding the specimen of *Z. tuerckheimii* as a young plant, it is easy to understand why it shows no pitted tracheids when we recall the writer's observation (**3**) that *Microcycas*, a rather slow-growing species with well developed trunk, shows a xylem ring with series of forty scalariform or transitional tracheids before the adult condition is established. In this connection, it may be recorded that the caudex of *Z. floridana* having diameter 130 mm., previously mentioned, shows a xylem ring 2.5 mm. in thickness, with approximately seventy-five tracheids in radial series, all scalariform with the exception of the protoxylem, which as usual is scalariform-reticulate.

One other case of a *Zamia* with trunk has been examined. In a letter to the writer, Dr. C. J. CHAMBERLAIN reports that in *Z. monticola*, a species with a stout trunk, he has observed bordered pits. This observation is entirely in accord with the writer's studies.

STRUCTURE OF PETIOLE

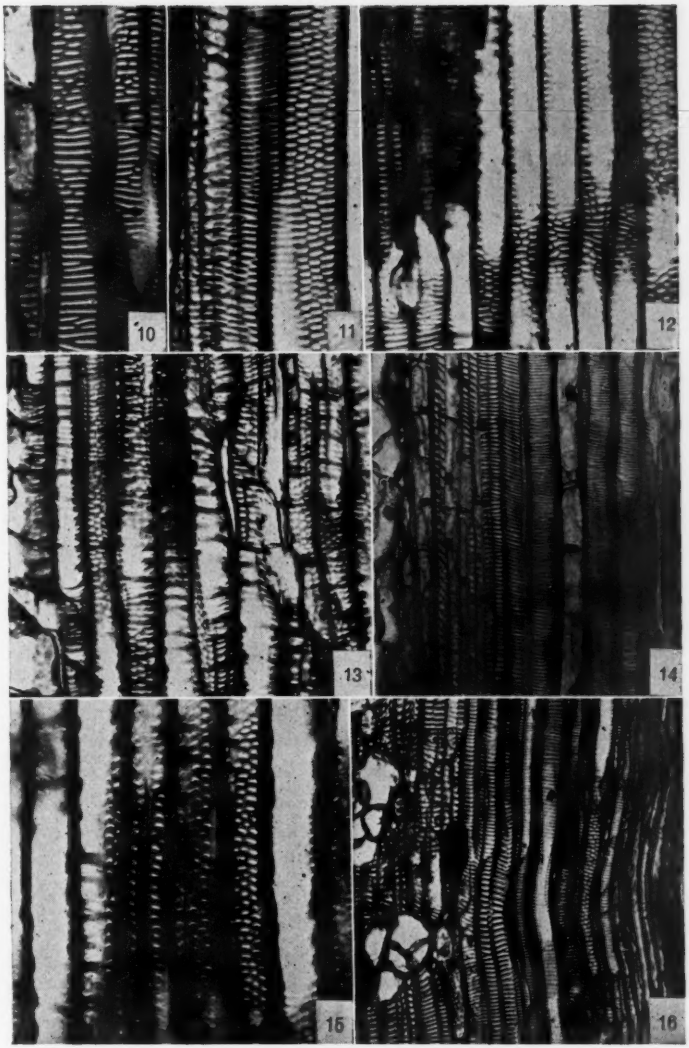
The observations on the tracheids of the petiole in *Z. floridana* have been extended to as many of the species of *Zamia* as have been available, namely, *umbrosa* Small, from Daytona, Florida; *latifoliolata* Prenl. and *kickxii* Miq., from Cuba; *furfuracea* Ait. (a Mexican species presented by the Director of the Cuba Experiment Station); *media* Jacq. (probably) from Puerto Rico through the kindness of Dr. M. T. COOK; an undetermined species from Hope Gardens, Jamaica; and the three treelike species from Central America. In each of these species the centripetal xylem shows tracheids ranging from spiral through scalariform to circular pitted, with elements intermediate between the last two. The pits are generally not in the numerous crowded series characteristic of genera such as *Ceratozamia* (see figure in BAILEY 1), but the circular pits are apt to occur mixed with slit-shaped ones and true scalariform openings on the same tracheid. Typical appearances are shown in the photographs of *Z. kickxii* (fig. 4), *furfuracea* (fig. 5), and *pseudoparasitica* (fig. 6). In many of the bundles, tracheids showing a condition beyond the transitional stage are scarce. It is of interest to note that the petiolar bundles of *Z. tuerckheimii*, a trunk-producing species, show well marked circular pits in some of the tracheids, although the one available stem presents only scalariform tracheids (*vide supra*).

The petiolar bundles of *Z. skinneri* present the further peculiarity of having ring-shaped thickenings as well as circular pits on some of the tracheids. This condition has already been noted as characteristic of the stem of this species, so that it appears to be a more or less specific feature, not restricted to *Z. skinneri* however.

Attention may be called to the abundance of pits on walls separating a tracheid from a parenchyma cell, as is shown in figure 3. The pits in this case are of course half-bordered.

PETIOLAR BUNDLES OF STANGERIA

The occurrence of circular pits in the leaf of *Zamia* spp. suggests an examination of *Stangeria*, the other genus having stem wood made



FIGS. 10–16.—Fig. 10, *Stangeria paradoxa*, transitional tracheids from centripetal xylem of leaf rachis. Fig. 11, same, spiral, scalariform, and pitted tracheids from leaf rachis. Fig. 12, *Zamia floridana*, secondary xylem of root showing transitional and pitted tracheids. Fig. 13, *Z. skinneri*, xylem of stem showing tertiary rings or spirals on pitted tracheids. Fig. 14, same, entire width of xylem of cone axis, with spiral and scalariform tracheids. Fig. 15, *Z. pseudoparasitica* showing xylem typical of root, with conspicuous tertiary spiral or ring-shaped thickening as well as bordered pits. Fig. 16, *Cycas revoluta*, xylem of cone axis with protoxylem at left, and pitted tracheid near phloem on right.

up of scalariform tracheids. Material has been available through the courtesy of the Director of the New York Botanical Garden. An average condition is shown in figure 10 and a more unusual one in figure 11, in which it is easy to make out the pitted condition, as well as transitional stages. Scalariform and spiral elements also occur toward the region of the protoxylem. Pits are most frequent in the outer or mature regions of the xylem mass, including the edges or flanks of a bundle; they are also abundant where a tracheid comes into contact with a row of parenchyma cells (*cf. Z. floridana*, fig. 3). These observations are at variance with the statement of MARSH (6), "no trace of ordinary bordered pitting was found anywhere in the plant." In his description of the petiolar bundles he states, "the metaxylem had pitting of a close scalariform type." This discrepancy may probably be due to the lack of uniformity in structure of different regions of the long rachis of the leaf in *Stangeria*.

BUNDLES OF CONE AXIS

SCOTT (7) showed that the peduncle of certain cycads possesses centripetal xylem, hence the reproductive axis has been regarded as a conservative organ. The xylem of the cone axis ought to throw light on the anomalous xylem of the tuberous species of *Zamia*. Cone axes of the following species have been available for study: *floridana*, *kickxii*, *media* (?), *pseudoparasitica*, *skinneri*, and an undetermined species (probably *gutierezii*) under cultivation in Cuba by Mr. H. A. van Hermann. In nearly all cases the bundles show a particularly clear series, beginning with narrow spiral or annular tracheids of the protoxylem (endarch), followed by wider spirals, scalariform tracheids, and finally phloem. This condition is illustrated in figure 14 for *Z. skinneri*. There is here no disturbing event such as the disruption of the primary xylem by growth of intruding parenchyma cells, characteristic of the stem, hence the recapitulation series is clear cut, although brief and incomplete on account of the absence of tracheids showing circular pits.

But the single cone axis of the Guatemalan *Z. tuerckheimii* which has been available shows the exceptional appearance of a series of well developed pitted and transition tracheids (fig. 9). This feature occurs in the peduncle, which appears aged or possibly wounded,

judging from the unusually dark color of the cell walls. The tracheids, moreover, are not so straight as usual, but appear more or less undulating. On account of the scarcity of material, it would be rash to do more than venture the suggestion that we may here see a traumatic reversion.

It has appeared desirable to extend the observations on peduncles to the other genera. In order to do this it has been necessary to enlist the services of the following, whose assistance in securing material of cones is most gratefully acknowledged: Dr. W. H. BROWN, Philippine Bureau of Science (*Cycas circinalis*, *C. rumphii*); Dr. C. J. CHAMBERLAIN, University of Chicago (*Dioon*); the late Dr. A. A. LAWSON, Sydney, N.S.W. (*Bowenia*, *Macrozamia*); Golden Gate Park, San Francisco (*Ceratozamia*, *Encephalartos*); Horticultural Park, Philadelphia (*Ceratozamia*, *Encephalartos*); New York Botanical Garden (*Stangeria*); Reasoner Brothers, Oneco, Florida (*Cycas revoluta*); Shaw Gardens, St. Louis (*Cycas circinalis*).

The results of this survey indicate that, so far as my material goes, in the genera *Dioon* (*edule*, *spinulosum*), *Ceratozamia*, *Encephalartos* (represented by *villosus*), *Macrozamia*, and *Microcycas* the condition is the same as that figured for *Zamia skinneri*, that is, the bundles of the peduncle show a series of narrow spiral, wide spiral, and scalariform tracheids, but nothing more advanced.

In *Stangeria*, however, the scalariform pits of the tracheids nearest the phloem show a tendency to become divided, thus constituting what we have called the transitional stage. Certain of the tracheids of this region, especially on the flanks of the bundles, show a reticulate appearance without distinct border to the meshes; but actual circular pits are practically absent in my material.

The cone axis of *Cycas revoluta* (microsporangiate, of course) goes a step further, having numerous tracheids with transitional markings and a few next the phloem with definite circular multiseriate bordered pits (fig. 16), thus exhibiting the complete recapitulation.

Finally, the cone axis of *Bowenia serrulata* shows numerous rows of circular pits, as well as slit-shaped pits and transitional stages. This observation is interesting on account of the tuberous habit of the stem in this genus.

That different species of a genus may vary in the extent of de-

velopment of the xylem is shown by the observation that in the cone axis of *Cycas circinalis* pits appear to be absent.

The incomplete recapitulation in the cone axis of many if not most of the cycads is not surprising in view of the short life of this organ, compared with the stem. The cone axis is continuous with the stem axis, and generally lays down a moderate thickness of secondary growth, say from fifteen to twenty-five rows of tracheids; but after the cone has begun to decay the cambium in the stem continues to produce tracheids, eventually reaching the condition of circular bordered pits. In fact, the only way in which a cone axis may produce pits is by hastening its recapitulation, and this appears to have taken place in *Bowenia*.

Structure of root

An examination of the root of *Zamia floridana* shows a structure surprisingly different from that of the stem. Instead of the uniform series of scalariform tracheids, the root presents an abundance of elements with circular and elongated pits, with many of the transitional elements (fig. 12). Any of these tracheids may be further strengthened by rather broad rings or spirals which may or may not show a definite tertiary origin. The appearance is hence much like that of the stem of the dendritic *Z. skinneri* (fig. 13).

The secondary xylem of the root of *Z. pseudoparasitica* (fig. 15) consists almost entirely of broad tracheids whose walls are covered with circular bordered pits, generally reinforced by the annular thickenings already noted as occurring in the root of *Z. floridana* and the stem of *Z. skinneri* (roots of the latter have not been available).

With respect to the other genera, roots of *Stangeria* have generously been supplied through the courtesy of the Director of the New York Botanical Garden. Although some of these roots have a diameter of 10 mm., their central cylinder shows but slight development, having only about sixteen rows of tracheids in the secondary xylem, all showing scalariform thickening. Apparently these roots are not so old as their diameter would indicate, and it yet remains to be seen whether an older root would show circular pits.

The genera having circular pits in their stems might be expected

to show them also in their roots. I have been able to check this point by examining roots of *Bowenia serrulata*, *Ceratozamia*, *Dioon spinulosum*, *Encephalartos villosus*, *Macrozamia denisonii*, and *Microcycas*. It is found that all of these show abundance of pits.

Discussion

The genus *Zamia* falls into line with the other genera with regard to the structure of its vascular elements. The scalariform condition, generally regarded as distinguishing this genus, appears to be restricted to the tuberous species. A survey of the various organs shows that circular pits are characteristic not only of the stem (in the trunk-forming species) but also of the root, leaf, and even of the cone axis in exceptional cases. It is impossible to avoid associating the scalariform tracheid with the tuberous habit, whether a complete survey of the genus would bear out this assumption or not. To reach a conclusion of such general import would involve as well a study of a number of members of the genera *Macrozamia* and *Encephalartos*, which share with *Zamia* the inclusion of tuberous as well as trunk-forming species. The requisite material is not now available. The only case which can be recorded at present is based on material kindly provided by Dr. C. J. CHAMBERLAIN. The stem of *Encephalartos altensteinii*, which "sometimes reaches a height of ten feet" (CHAMBERLAIN), shows an abundance of circular pits in its tracheids; while *E. brachyphyllus*, a strictly tuberous species from Zululand, shows nothing more advanced than scalariform markings.

It is submitted that the evidence so far accumulated points to the inference that the tuberous species of *Zamia* have literally never "grown up," that is, they are persistent juveniles in habit and in some of their vascular tissues. That they are not completely immature is obvious when one recalls the group of strobili borne by an average plant of *Z. floridana*. The ancestors of the modern *Zamia* species may be pictured as trees on account of the tropical range of the family. Perhaps the tuberous species represent members of the genus that have met evil days in respect of climate, or an extension of the genus into less favorable climatic regions; *Z. floridana* lives in calcareous sand, freely exposed to the sun, while *Z. kickxii* of western Cuba has its caudex buried in exceedingly hard and unfriend-

ly soil. On the other hand, the trunk-forming species of Central America are reported to occur in montane or coastal rain forest. Moreover the stiff thick leaflets of these tuberous species are in marked contrast to the broad and thinner leaflets of *Z. pseudoparasitica*. But it would be unwise to press this point too far, as measurements of leaf thickness in a number of species show. And it will be recalled that some trunk-forming cycads (for example, *Dioon*) live in an exceedingly dry habitat. WIELAND (9) has recorded his mature opinion that the cycadeoids of the Cretaceous, which at best have a short trunk, represent a climax of xerophytic plants.

But for one reason or another, the tuberous species of *Zamia* retain a juvenile habit and xylem. There are other better known plants which are persistently juvenile in habit, for instance the so-called *Retinispora* species, which are regarded as members of the genus *Chamaecyparis* or *Thuja*. These ornamental conifers retain the juvenile foliage for an indefinite period and are usually sterile. Occasionally, however, seed cones are produced, as has been observed in New Brunswick during 1936 in the case of *R. pisifera*. In the Royal Gardens at Kew is a group of large specimens of a juvenile form of *Cryptomeria*. The seedling foliage of *Thuja* is a familiar object in northern swamps, and the mixture of juvenile and mature branches of *Juniperus virginiana* will also be recalled. COCKAYNE (4) remarks that in New Zealand no fewer than 200 species of spermatophytes pass through a juvenile stage. This is especially well marked in *Dacrydium* and *Podocarpus*, and may persist for as long as eighty years in *P. dacrydioides*.

The monotypic *Stangeria* appears to illustrate the same juvenile features as *Zamia* spp. This is a particularly interesting case on account of the primitive features shown by the leaf. It is a matter of common knowledge that when the plant was first grown at Kew it was mistaken for a fern. But the xylem of the stem is not primitive, it is immature, or in other words its development has been arrested, as is indicated by the occurrence of pitted tracheids in the leaf and cone axis.

It would be indeed strange if the xylem of present day cycads were not pitted, in view of the fact that many Paleozoic plants (for example, *Lyginopteris* and *Cordaites*) had at that early period reached

the stage of a tracheid with crowded circular pits. Longitudinal sections through the stem of *Lyginopteris* show circular pits in the mesarch leaf traces as well as the central cylinder of the stem. Somewhere among these plants must be the ancestors of our modern cycads.

The present observations on the tracheids of cycads are summarized in table 1. The prevalence of circular pits in the group is obvi-

TABLE 1

NATURE OF PITTING IN XYLEM OF CYCADS, REPRESENTING MOST ADVANCED CONDITION REACHED

MATERIAL	HABIT	STEM	ROOT	PETIOLE	CONE AXIS
Bowenia serrulata	Tuber	Pitted	Slit-shaped	Pitted	Pitted
Ceratozamia mexicana	Trunk	Pitted	Pitted	Pitted	Scalariform
Cycas revoluta	Trunk	Pitted	Pitted	Pitted	Scalariform and transitional
Cycas circinalis	Trunk	Pitted	Pitted	Pitted	Scalariform
Dioon spinulosum	Trunk	Pitted	Pitted	Pitted	Scalariform
Encephalartos villosus	?	?	Pitted	Pitted	Scalariform
Macrozamia spiralis	?	Pitted	?	Pitted	Scalariform
Macrozamia denisonii	Trunk	?	Pitted	?	?
Microcycas	Trunk	Pitted	Pitted	Transitional	Scalariform
Stangeria	Tuber	Scalariform	Scalariform?	Pitted	Transitional
Zamia floridana	Tuber	Scalariform	Pitted and rings	Pitted	Scalariform
Zamia kickxii	Tuber	Scalariform	?	Pitted	Scalariform
Zamia pseudoparasitica	Trunk	Pitted	Pitted and rings	Pitted	Scalariform
Zamia skinneri	Trunk	Pitted and rings	?	Pitted	Scalariform

ous. Their relative scarcity in the cone axis also is noticeable. The differences shown by the xylem of the various organs may be regarded as so many cases of recapitulation of the phylogenetic development of the tracheid from steep spiral to circular pitted.

STEM.—In most members of the group there is a complete and gradual recapitulation in the stem. For instance, it has been observed (3) that in *Microcycas* a series of approximately forty tracheids is formed before the pitted condition is fully established, and this number does not include the protoxylem which in the stem is

disrupted by intrusion of parenchyma. In *Stangeria* and the tuberous *Zamia* spp., however, the ontogeny is incomplete or arrested as well as gradual.

CONE AXIS.—In most genera the cone axis also illustrates an incomplete recapitulation which, however, is regular on account of a lack of disturbance in the region of the primary xylem. Some genera show a complete recapitulation, for example, *Bowenia*, which is nevertheless a genus with tuberous stem. In all cases which have been observed, the stages between steep spiral and scalariform are passed over rapidly, in contrast with the deliberate habit of the stem. The case of *Cycas* is of special interest. We have become accustomed to think of this as a particularly primitive genus, a "living fossil," on account of the fact that its megasporophylls are not aggregated into a cone but are distinct and leaflike. But the microsporophylls show no such primitive features, either external or internal, for the xylem illustrates a complete recapitulation and has little or no sign of centripetal growth. Perhaps, however, we should regard a complete recapitulation as nearer the ancestral condition than is the incomplete one. (A more extended treatment of this case is in preparation.)

ROOT.—The root appears to pass rapidly through the early phylogenetic stages, for the secondary xylem in most of the genera so far examined consists of pitted or transitional tracheids. It might be expected that in an organ so shielded from external factors of an extreme nature the primitive features would persist, but such does not seem to be the case. At all events the abundant pitted tracheids of the root repeat the condition characteristic of what are believed to be the ancestors of the cycads.

LEAF.—The leaf differs from the other organs in the almost complete replacement (except at the base of the petiole) of the usual centrifugal by a group of centripetal tracheids. In all of the genera this group exhibits a rapid progression from normal protoxylem to pitted metaxylem, that is, the recapitulation in most genera is complete but condensed. In *Stangeria* and most species of *Zamia*, however, the series hardly passes beyond the transitional stage, although circular bordered pits have been shown to occur. Since all of the centripetal xylem is primary, the completeness of the recapitula-

tion is worthy of remark. In none of the cycads can be observed that overlapping or telescoping of primitive and advanced elements which BAILEY (1) has described for conifers.

Summary

1. An explanation is offered for the apparently anomalous composition of the xylem of the stem in *Zamia* and *Stangeria*.

2. It is shown that although the stem wood of tuberous species of *Zamia* does not pass beyond the scalariform stage, certain trunk-producing species from Central America show abundance of tracheids with circular bordered pits.

3. The presence of circular pits in the tracheids of the leaf rachis is hence explained; otherwise the occurrence of pits would constitute an exception to the doctrine of conservative organs.

4. The tuberous species of *Zamia* are regarded as persistent juveniles with respect to their growth habit and their xylem; that is, they remain immature vegetatively although they reproduce freely.

5. A comparison is made between these juvenile cycads and such persistent juveniles as the *Retinispora* species and other conifers.

6. A similar explanation is considered to apply to *Stangeria*.

7. All organs of the cycad plant are surveyed in order to test the validity of the explanation, and the differences shown by the xylem in stem, cone axis, root, and leaf are interpreted in terms of relative completeness and rapidity of the recapitulation which the different organs exhibit in the various genera. The stem exhibits a complete although gradual recapitulation, incomplete, however, in the tuberous species of *Zamia*; the reproductive axis has a much more condensed series, generally incomplete; the root quickly passes through the early phylogenetic stages; finally the leaf shows in its centripetal xylem a much abbreviated recapitulation, which tends to be incomplete in the tuberous members of the group.

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SEEDLING ANATOMY OF CYNARA SCOLYMUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 479

WALTER S. PHILLIPS

(WITH TWENTY-TWO FIGURES)

Introduction

According to DE CANDOLLE (2) *Cynara scolymus* L., the Globe artichoke, is a native of the Mediterranean region. MORIS, DE CANDOLLE reports, cultivated *C. cardunculus* and *C. scolymus* side by side and found no true specific distinctions. He therefore applied the name *C. cardunculus* var. *sativa* to *C. scolymus*. STURTEVANT (14) discussed the synonymy and history of this species. At the present time the most important area for commercial production in the United States is along the California coast, between Santa Barbara and San Francisco, where about 9000 acres are devoted to its cultivation (11).

Little morphological work has been done with *Cynara scolymus*, and this has dealt chiefly with the chemical contents of the flower heads, which constitute the commercial part of the plant. OKEY and WILLIAMS (9) reported inulin as the chief carbohydrate, with a small percentage of reducing sugars also present. SCOTT (11) discussed propagation briefly and also included chemical analyses of the subterranean and aerial parts of the plant. LE FEUVRE (8) and WELLINGTON (19) investigated propagation and cultivation. JEFFREY (6) considered the tribe Cynareae as "transitional anatomically from the Tubuliflorae to the Liguliflorae," and regarded the oil canals found in *C. scolymus* as primitive structures in what he considered to be the more stable parts of the plant. SACHS (10) and VAN TIEGHEM (18) described the oil canals or ducts, which are of schizogenous origin and occur between the two layers of the endodermis. These have been reported as occurring in many members of this family, according to SOLEREDER (13).

Material and methods

The seeds used in this investigation were obtained from the Bureau of Foreign Plant Introduction at their Chico, California, sta-

tion, and also from a commercial seed company. Three varieties, Green Globe, Flat Brittany, and Large Globe or Paris Improved, were found to be practically identical in their seedling anatomy. The straightest seedlings were grown on moist paper in tumblers, but in order to check their development with seedlings grown in soil, akenes were also planted in soil and serial sections of these plants were compared with those germinated on paper. No morphological differences were found.

Navashin's solution and chrom-acetic solutions proved the best fixatives. After fixing, the material was washed for several hours in warm water to soften and dissolve the inulin as much as possible. Serial sections were cut 8–15 μ in thickness and stained with Flemming's triple stain or with safranin and fast green. The safranin-fast green stain was very satisfactory in making drawings with microprojection apparatus.

Investigation

Cynara scolymus is propagated by suckers or by seed. The sucker method is usually followed because it brings the plants into commercial production sooner. The plant is a perennial with the rosette habit, the flower stalk not appearing until the second year in plants grown from seed. As soon as the flower stalk dies back, about a dozen new shoots develop from axillary buds of the rosette leaves. SCOTT reports that these new shoots develop an adventitious root system and that the old primary root becomes fleshy and functions as a storage organ.

The fruit is an akene containing a single erect anatropous ovule. Endosperm is lacking and the nucellar tissue consists of a thin membranaceous layer of cells closely adhering to the ovary wall. The small hypocotyl and epicotyl lie at the basal end of the ovary, and the two thick cotyledons fill the remainder.

At germination the primary root pushes through the coats of the akene by the second or third day. Rapid elongation of the primary root takes place first; then the hypocotyl elongates and raises the cotyledons, partially inclosed in the akene, above the ground. The cotyledons expand rapidly and function as fleshy photosynthetic

leaves for several days before the first epicotyledonary leaves appear. The mature cotyledons are obovate, tapering slightly toward the thick fleshy petioles.

Primary root

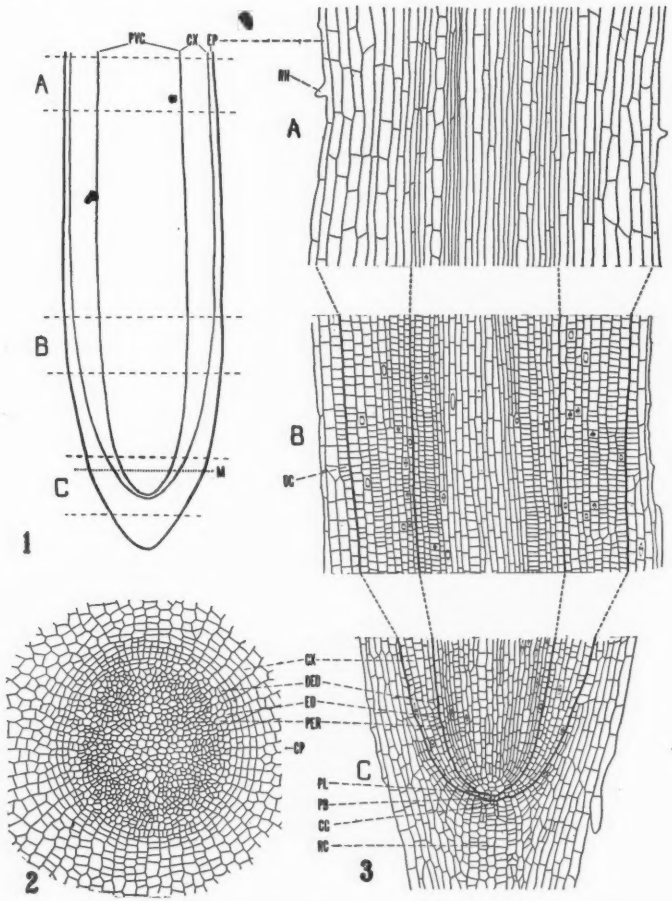
The primary root is diarch, with the four or five protoxylem elements of each point alternating with two lateral bands of phloem cells. Jeffrey (6) has figured the primary root as triarch, but in the seedlings examined this condition was never encountered. Some of the adventitious roots of older plants were tetrarch. Parenchymatous cells separate the phloem from the xylem in the region where the cambium is later to develop, and there are several large parenchymatous cells in the central region of the stele. This whole cylinder of vascular tissue is surrounded by the pericycle, which is at first uniseriate. The primary xylem elements remain intact after secondary thickening has taken place, and are clearly discernible in the mature root.

The endodermis limits the cortex centripetally, but Casparian strips are not developed until after there has been some secondary thickening. The endodermis can be distinguished from the other cortical cells by the early development of primary endodermal oil ducts, which accurately locate the endodermal layers even before the formation of the Casparian strips. The layers of cortical cells adjacent to the endodermis have a radial arrangement which is lost in the outer limits of the cortex. The cortex persists intact for a considerable time, but after secondary thickening of the axis has started, a phellogen is formed midway between the endodermis and the epidermis and the outer cortical cells and epidermis slough off.

The development of the primary root axis is similar to that described by Janczewski (5) for *Helianthus annuus*. It follows his third type, in which plerome and periblem produce stele and cortex respectively and a common layer initiates both the epidermis and calyptra.

This common layer of cells overlying the periblem has been termed the calyptrogen-dermatogen by Crooks (3) because both the root cap and the epidermis are derived from it. Eriksson (4) has referred to it as the dermacalyptrogen. This layer divides in a series of periclinal divisions at the apex of the root, giving rise to the more

or less radially arranged cells of the terminal portion of the root cap (figs. 1, 3*C*). Periclinal divisions also occur in the lateral por-



FIGS. 1–3.—Fig. 1, diagrammatic representation of root tip. *A*, *B*, and *C* represent areas shown in detail in fig. 3. *M*, position of cross section shown in fig. 2. Fig. 2, cross section through meristem of root at level where endodermal layer divides. Fig. 3, *A*, *B*, *C*, longitudinal sections of root. *cg*, calyptrogen; *cp*, cotyledonary plane; *cx*, cortex; *ded*, double endodermis; *dg*, dermatogen; *ed*, single endodermal layer; *ep*, epidermis; *pb*, periblem; *per*, pericycle; *pl*, plerome; *rc*, root cap; *rh*, root hair.

tions of this layer, where anticlinal divisions are taking place. In this manner the root cap becomes an elongate structure extending some distance upward from the tip of the root, but being thickest at the apex. The lateral portions of this layer form the epidermis by a series of anticlinal divisions (fig. 3*B*).

The cortex is derived from a single layer of cells, the periblem, which lies directly inside the calyptrogen-dermatogen layer. The endodermis, which limits the cortex on the inside, is a cylindrical layer of tissue two cells in thickness, with structural features unlike those of the cells on either side. This double layer of cells originates from the innermost layer of the periblem near the apex of the root by a single periclinal division (figs. 2, 3*C*). The cytoplasm in the endodermal cells is dense and contains no inulin crystals. Oil ducts develop by the formation of schizogenous intercellular spaces between these two layers of endodermal cells. These are not formed where the endodermis lies exterior to the protoxylem points. BARY (1) considers corresponding cell layers in other plants as endodermal but in this case only the layer next to the stele has been found to develop Casparian strips. These ducts have been described by SACHS (10) and VAN TIEGHEM (18). SOLEREDER (13) summarizes the work on oil ducts done in this and other groups of plants.

The plerome gives rise to the stele and consists of a small group of cells directly within the periblem. The larger parenchymatous cells at the center of the axis are discernible at an early stage in the differentiation of the stele. The pericycle, which is at first a single layer, becomes several cells thick by means of tangential divisions of its cells (fig. 21). The first vascular tissue to differentiate is the primary phloem, which is formed by divisions in a vertical plane of the plerome cells immediately within the pericycle. Six primary phloem ducts, three on either side of the cotyledonary plane, are at first evident at the outside limits of the phloem, but these are soon crushed by the enlargement of cells which differentiate as sieve tubes and companion cells. The primary xylem is differentiated centripetally. The protoxylem elements have annular-spiral thickenings, and the metaxylem scalariform thickenings.

PROVASCULAR SEEDLING ANATOMY

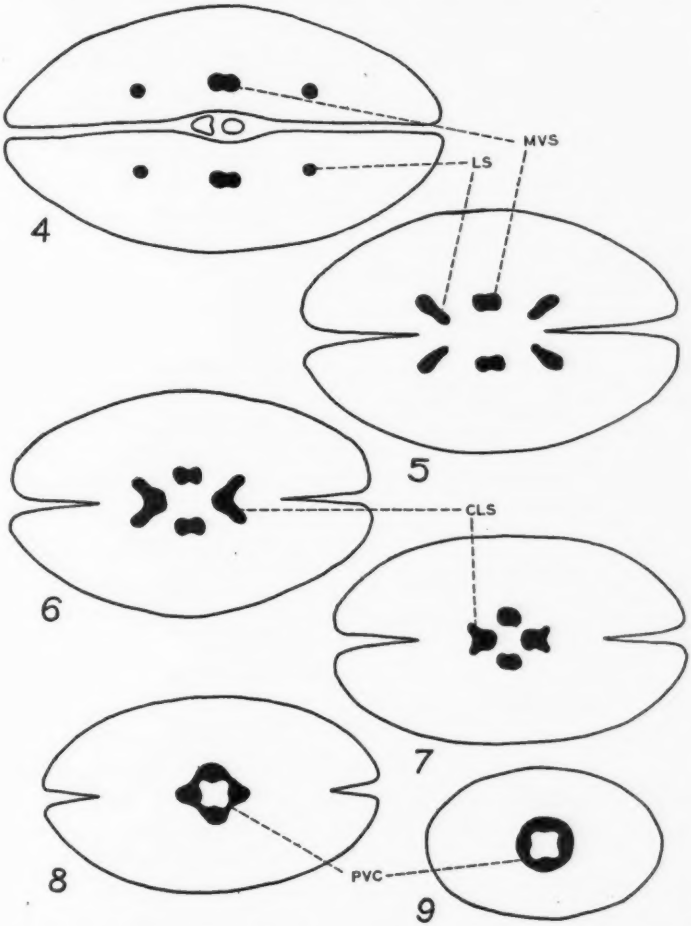
In a day-old seedling the provascular strands can easily be traced and the ultimate arrangement of the vascular system is clearly indicated.

In each cotyledon there is a median strand of provascular tissue flanked on either side by one or more smaller lateral strands (fig. 4). The number of laterals in the cotyledons varies, one or two being the usual number, although some cotyledons have been observed with one lateral strand on one side of the midvein and two on the other. In cross section the provascular strand of the midvein appears lobed, while the lateral strands are circular. The cells of the mesophyll region of the cotyledon are filled with large inulin sphaerocrystals, which take a deep stain, while the cells of the provascular strands are not so large and are not filled with these large crystals but contain a minutely granular substance which does not stain deeply.

At this age the phloem and xylem regions are not differentiated, but a little later in the ontogeny primary phloem ducts are differentiated, and concurrently with the formation of these ducts the first annular protoxylem elements are matured. The initial differentiation of vascular tissue occurs near the base of the cotyledonary petiole and proceeds both upward and downward. This development differs from that of the root in that the protoxylem of the root does not differentiate until after the primary phloem is well defined.

In tracing the provascular strands downward from the cotyledons through the hypocotyl to the meristematic region of the root, they remain separate to a level just below the point of divergence of the cotyledons. At this level the lateral strands occupy a more central position (fig. 5), and still lower in the hypocotyledonary axis the lateral strands from opposite cotyledons converge (fig. 6). This results in the formation of two strands in the cotyledonary plane on opposite sides of the axis of the hypocotyl (fig. 7). When there are two lateral strands on each side of the midvein, the outermost strands of each cotyledon converge to form a common strand, with their counterpart in the opposed cotyledon, and these in turn anastomose with the common strand of the inner laterals of each cotyledon (fig. 22).

The midvein strands, which appear lobed in cross section in the cotyledons (figs. 4–6), become ovate in outline near the base of the cotyledons (fig. 7). The two median strands then converge in the hypocotyl with the united lateral strands to form the fluted central



FIGS. 4–9.—Cross sections through young embryo. Provascular strands shown in black. *cls*, united lateral strand of opposite cotyledons; *ls*, lateral strand; *mvs*, midvein strand; *pvc*, provascular cylinder.

provascular cylinder (fig. 8). The lobed condition of this cylinder is less evident at lower levels (fig. 9) and the stele becomes circular in cross section. This cylinder of cells has at its center larger cells (fig. 2) whose cytoplasmic contents are less dense than those surrounding them.

VASCULAR ANATOMY OF HYPOCOTYL AND COTYLEDONS

The course of the vascular bundles of the cotyledons and hypocotyl is shown graphically (fig. 22). In the following description the bundles are traced from their point of initiation in the cotyledons to the diarch root. To avoid confusion, the median bundle is first discussed and then the course of the lateral bundles of the cotyledons is considered.

About midway between the base and the tip of the cotyledon of a three- to ten-day old seedling, there have been differentiated two vascular bundles which constitute the midvein. The two protoxylem strands of each bundle lie in close association with each other and the metaxylem is tangentially oriented with respect to the protoxylem (fig. 16). The phloem of each bundle lies in an abaxial position in relation to each metaxylem group. These two closely associated bundles are surrounded by a sclerenchymatous sheath of cells whose walls are distinctly thicker than those of the mesophyll cells.

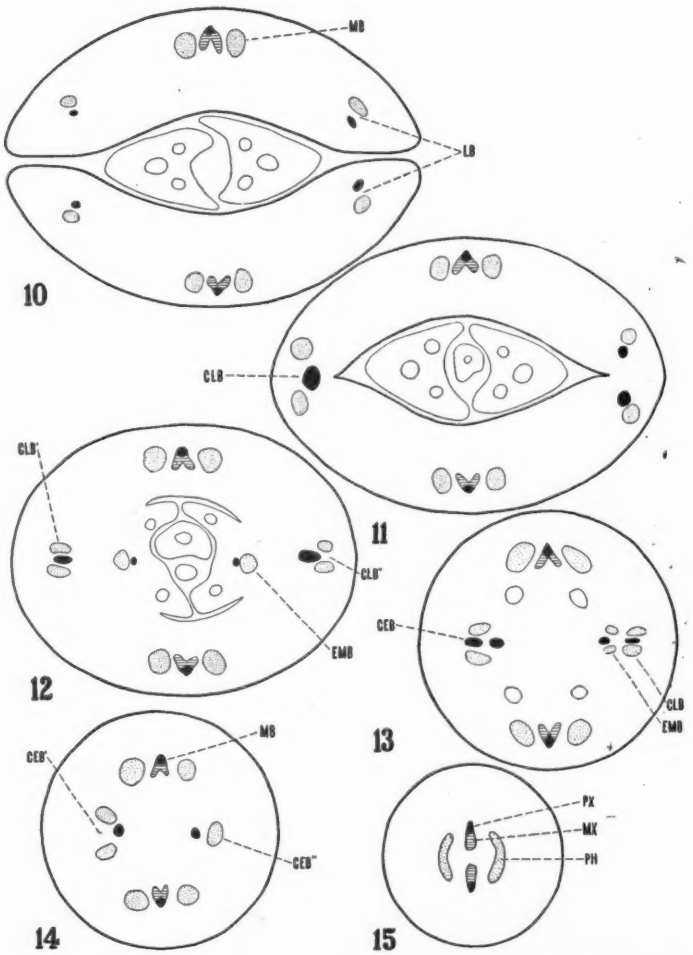
THOMAS (**16, 17**) discusses the double bundle, which sometimes constitutes the midvein of the cotyledons, in relation to types of transition found in certain seedlings. The double bundle is reported as consisting of two groups of primary phloem with a single strand of protoxylem between, the three groups being in radial arrangement; but no mention is made of the metaxylem of this bundle. In *Cynara scolymus* the metaxylem is very evident and the midvein is truly a double bundle located along the median line of the cotyledon and consisting of two separate strands, each of which contains protoxylem, metaxylem, and phloem. The vascular elements of these two distinct bundles, however, are inclosed in a common sheath (fig. 16). The two protoxylem strands unite near the base of the cotyledon, in the petiole, so that the bundle contains two groups of phloem, two groups of metaxylem, but only one common protoxylem group. At a lower level, at the base of the hypocotyl, the metaxylem

groups also unite, but the two phloem strands of each double bundle never unite to form a common strand. Instead one phloem strand on each side of the median bundle of one cotyledon unites with the equivalent phloem strand of the opposed cotyledonary median bundle to form the lateral phloem strands of the diarch root. The interpretation of the median bundle as a double one depends, therefore, upon the level at which sections are taken.

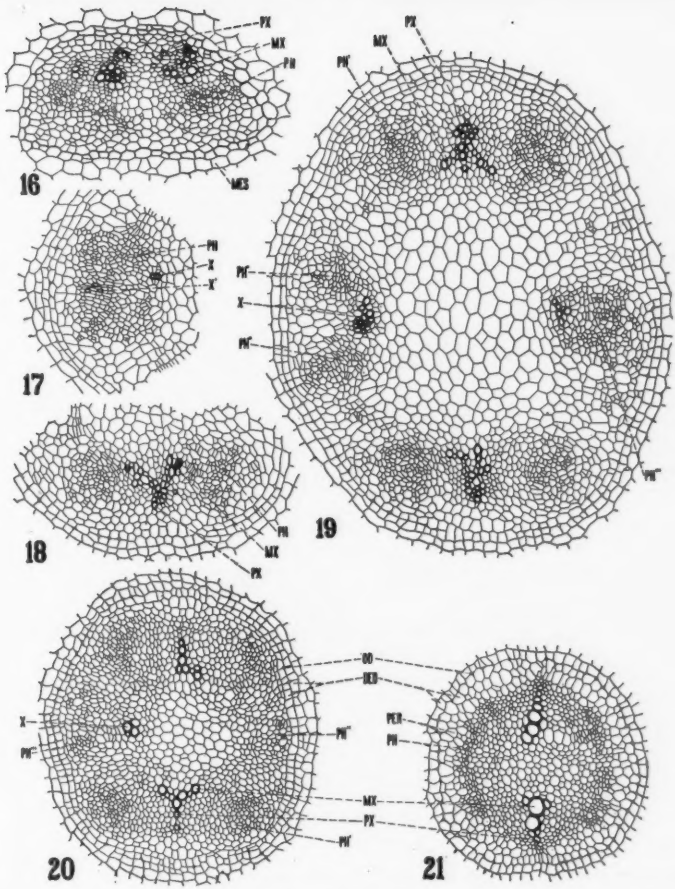
At the base of each cotyledon and below the point where the protoxylem strands of the double bundle have converged, there is formed a bundle which has the primary xylem in a V-shaped arrangement. The protoxylem lies in an abaxial position at the apex of the V and the two metaxylem groups form the arms. The strands of primary phloem lie on either side of the metaxylem (fig. 10*mb*). This arrangement of the median bundle is also found in the upper part of the hypocotyl (fig. 18). At successively lower levels (figs. 19, 20) in the hypocotyl the differentiation of metaxylem with reference to the protoxylem is more and more centripetal, which brings the two arms of metaxylem into closer association. The two strands of phloem still lie in a lateral position with reference to the primary xylem (fig. 14*mb*). At the base of the hypocotyl the primary xylem differentiates in the exarch manner of the root (fig. 21), and the two groups of phloem on each side of the intercotyledonary plane converge, forming a single strand of phloem on either side of the primary xylem (figs. 15, 21).

The lateral bundles of the cotyledons are endarch collateral (fig. 10*lb*), but at a level just below the divergence of the cotyledons they converge toward the cotyledonary plane, and the xylem of the lateral bundles of opposite cotyledons unites (fig. 11*clb*). This fusion bundle then consists of xylem flanked on either side, parallel to the intercotyledonary plane, by phloem (fig. 12*clb'*). The phloem becomes reoriented at a lower level (figs. 12*clb''*, 14*ceb'*) and finally lies outside of the xylem and on the same radius (fig. 14*ceb''*). Ultimately this vascular unit ends blindly in the lower hypocotyl (fig. 20).

LEE (**7**) and SILER (**12**) both describe transitions very similar to that described here for *Cynara scolymus*. LEE discusses the transition of *Arctium majus*, while SILER discusses a like transition for *A. minus*. The main facts of the transition in these two species con-



FIGS. 10–15.—Diagrammatic representation of vascular anatomy of three- to ten-day old seedling. Primary xylem shown by black; metaxylem, lined; primary phloem, stippled. *ceb*, bundle composed of midvein of ~~epicotyledonary~~ leaf and combined lateral veins of opposed cotyledons; *clb*, combined lateral bundle of opposite cotyledons; *emb*, midvein of ~~epicotyledonary~~ leaf; *lb*, lateral bundles of cotyledons; *mb*, median bundle; *mx*, metaxylem of root; *ph*, primary phloem; *px*, protoxylem of root.



FIGS. 16–21.—Cellular details of transition and root: Fig. 16, structure of double bundle of midvein of cotyledon. *ph*, phloem; *px*, protoxylem; *mes*, mesophyll; *mx*, metaxylem. Fig. 17, epicotyledonary bundle converging with lateral cotyledonary bundle. *ph*, phloem of both bundles united; *x*, xylem of epicotyledonary leaf; *x'*, of lateral cotyledonary bundle. Fig. 18, cotyledonary midvein bundle in upper part of hypocotyl. Fig. 19, middle of hypocotyl. *ph'*, phloem of bundle in cotyledonary plane; *ph''*, in intercotyledonary plane; *x*, xylem of intercotyledonary bundle; *ph'''*, phloem of intercotyledonary bundles united. Figs. 20, 21, section at base of hypocotyl and root respectively. *ded*, double endodermis; *od*, oil ducts; *per*, pericycle; *ph*, phloem of root; *ph'*, of bundle in cotyledonary plane; *ph''*, in intercotyledonary plane (xylem has not differentiated); *ph'''*, phloem of opposite bundle; *x*, xylem of bundle in intercotyledonary plane.

form to those found in *C. scolymus*. The merging of the lateral cotyledonary bundles with the metaxylem of the cotyledonary midvein bundle which they describe for these species is not encountered in *C. scolymus*. Instead in the young seedlings these lateral bundles end

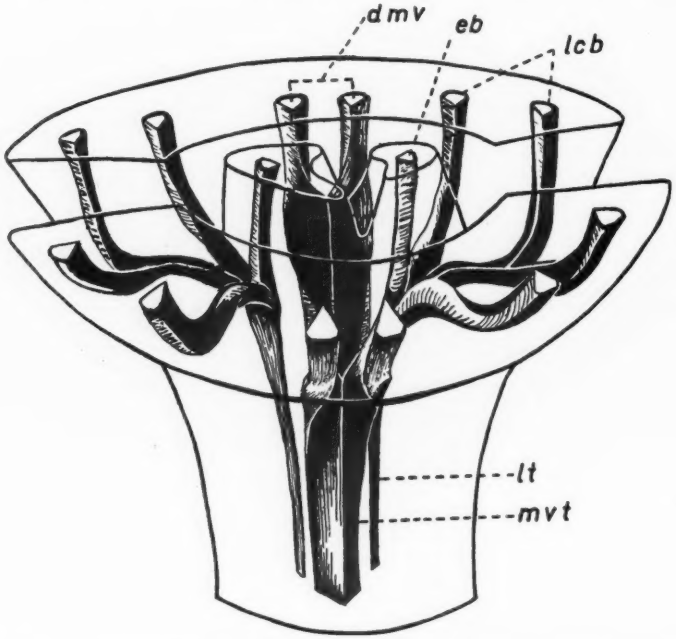


FIG. 22.—Schematic diagram of seedling vascular system showing two laterals on each side of midvein bundle. *eb*, epicotyledonary midvein bundle; *lcb*, lateral cotyledonary bundle; *lt*, lateral bundle of intercotyledonary plane; *mvt*, bundle of cotyledonary plane; *dmv*, double midvein bundle.

blindly in the hypocotyl. The only connection between these lateral bundles and the primary vascular elements of the root is by means of secondary tissues which are laid down by the cambium later in the ontogeny of the axis.

VASCULAR SYSTEM OF EPICOTYL

In a three- to ten-day old seedling the first two foliar leaves have developed provascular tissue consisting of a median bundle with a

lateral bundle on either side. At about the level of divergence of the cotyledons, the median bundle of the epicotyledonary leaf has differentiated as a collateral endarch bundle consisting of protoxylem and phloem (fig. 12*emb*). At a slightly lower level the phloem differentiates as two strands, which come to lie on either side of the xylem in a plane parallel to the cotyledonary plane (fig. 13*emb*). This midvein trace extends downward, and at about the middle of the hypocotyl unites with the cotyledonary trace, which lies in the intercotyledonary plane. The phloem strands of the combining bundles unite at a higher level (figs. 13*ceb*, 17) than do the xylem strands. After the xylem of the two bundles unites, the combined bundle consists of xylem flanked on either side by phloem. Near the base of the hypocotyl this bundle forms a collateral bundle by the convergence of the phloem strands (fig. 14*ceb'*, *ceb''*).

Summary

1. Germination of the seed and development of the seedling of *Cynara scolymus* are discussed.

2. The embryo shows well defined provascular strands which later differentiate vascular tissue.

3. The primary root is diarch with three clearly defined histogens. The plerome and periblem give rise to the stele and cortex respectively, while the root cap and epidermis are derived from a common histogen, the calyptogen-dermatogen.

4. The order of differentiation of the primary vascular elements is described.

5. The primary endodermal oil ducts arise schizogenously between the layers of the double endodermis.

6. In the transition, the primary xylem of the root in the cotyledonary plane is continuous with the xylem of the midveins of the cotyledons. The lateral bundles of the cotyledons end blindly in the intercotyledonary plane of the hypocotyl.

7. The structure of the double bundle of the cotyledon is described.

8. The midveins of the first foliar leaves anastomose with the lateral bundles of the cotyledons in the hypocotyl.

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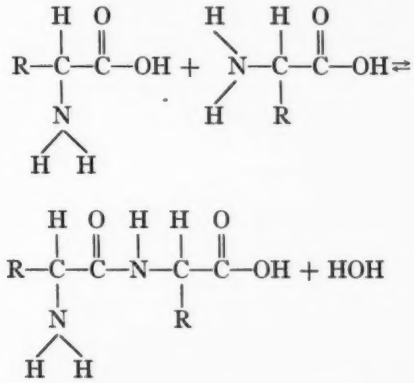
POTASSIUM AND CALCIUM IN RELATION TO NITROGEN METABOLISM[1]

G. T. NIGHTINGALE

In both plant and animal metabolism, the "protein sparing" action of carbohydrates has long been known and in plants has been repeatedly demonstrated by the experiments of SCHULZE and PRIANISCHNIKOV (15). As long as carbohydrate reserves are high there tends to be immobilization of organic nitrogenous reserves. With increase in content of dry matter there is loss of water from amino acids and condensation to polypeptides and insoluble proteins, thus:



Conversely, with decrease in carbohydrates, proteins are hydrolyzed to relatively simple, water soluble, mobile forms of organic nitrogen, which under favorable conditions are readily available for the synthesis of the proteins of meristematic tissue: proteins very different in quality from the storage proteins which are apparently the condensation products of amino acids. The cyclic structure hypothesis

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may perhaps lead to a better understanding of the nature of the proteins of meristematic structures, for it indicates that amino acids can enter into combinations that are not included in the conventional peptide hypothesis, which, as already pointed out, is primarily that of synthesis of proteins through chemical dehydration of amino acids.

In apparent contrast to the immobilization of organic nitrogenous reserves under conditions of carbohydrate accumulation, there is the work of MASON and MASKELL (9) on translocation of nitrogen in nitrogen deficient plants, that of MURNEEK (11) on autumnal migration of nitrogen from apple leaves, and that of RICHARDS and TEMPLEMAN (18) on transport of nitrogen from the lower leaves of nitrogen deficient barley plants. RICHARDS and TEMPLEMAN take exception to the general ideas expressed, on the basis that, under conditions of shortage of nitrogen, the plants should not be expected to store the limiting element. This is a convenient explanation but means little. Certain it is that if nitrogen deficient, high carbohydrate plants are shaded, shifted to darkness, or heavily pruned, there is, accompanying the decrease in carbohydrates, very rapid hydrolysis of proteins and re-utilization of the resulting amino acids to form new proteins of the meristematic regions. This is accompanied by a striking increase in volume of the plant even though there is no external supply of nitrogen available.

As MOTHES (10) shows, however, the several results mentioned are not necessarily in conflict. His extensive studies of the nitrogen metabolism of leaves fully corroborated the earlier work of SCHULZE and PRIANISCHNIKOV on the "protein sparing" action of carbohydrates (15). In addition, MOTHES found that old leaves (as the primary leaves of bean, even before they showed any external signs of senescence) exhibited proteolysis both on increase of carbohydrates in the light and on addition of glucose in the dark. Regardless of carbohydrate content, undoubtedly there is cleavage of protein as tissues approach senescence, and this often occurs in the more advanced stages of nitrogen deficiency and in maturing leaves. As a general thing, however, carbohydrate accumulation in plants is characterized by condensation and immobilization of organic nitrogenous reserves, and decrease in carbohydrates is correlated with

hydrolysis of stored proteins. Under favorable conditions the development of new tissues accompanies this hydrolysis of stored proteins.

The preceding remarks indicate in a general way the course of metabolism of organic nitrogenous materials already in storage in the plant. There are various external sources of nitrogen which plants can utilize, but under usual commercial field conditions the principal external source of nitrogen is undoubtedly nitrate.

In many plants nitrate may be stored in enormous quantities. Not infrequently nitrate in plant tissues may accumulate until a concentration is reached that is equal to or even greater than that of the organic nitrogen. However, the greatest storage of nitrate usually occurs, not under conditions most favorable for active protein synthesis, but under conditions which are less favorable, assuming of course an abundant external supply of nitrate.

Although all cell constituents necessarily exert an effect upon the plant, the presence of nitrate in itself will not result in the formation of new cells and growth. There must first occur reduction of nitrate to nitrite, then to ammonium, and eventually organic nitrogen synthesis. This involves an endothermic reaction with oxidation of carbohydrates or their derivatives and a greatly increased rate of respiration, as recently demonstrated by HAMNER (**6**). Clearly, new synthesis of organic nitrogen, if carried to excess, will tend greatly to deplete the carbohydrate reserves of the plant, especially under conditions of limited opportunity for carbon dioxide assimilation.

In connection with student instruction and in formal research experiments (**12, 13, 14**), the writer has had the opportunity, over a period of about ten years, repeatedly to observe effects of potassium and calcium deficiency on the growth and nitrogen metabolism of several different species of dicotyledonous plants.

POTASSIUM DEFICIENCY.—When plants are abundantly supplied with nitrate under conditions reasonably favorable for carbon dioxide assimilation, one of the first effects of lack of potassium is invariably that of nitrate and carbohydrate accumulation. Accompanying the increase in carbohydrates there is immobilization of amino acids. Externally the plants exhibit all the general symptoms usually included by the term nitrogen deficiency. "Leaf scorch,"

frequently exhibited by fruit trees (19), and various other symptoms sometimes considered to be more or less characteristic of lack of potassium, may be induced or very largely eliminated by varying the supply of other nutrient ions or the opportunity for carbon dioxide assimilation. During early stages the situation is also anatomically much the same as that of plants lacking an external nitrogen supply. Mechanical elements are strongly developed, but actively dividing cells are few and limited to apical tissues, which are the only regions where potassium can be found in quantity in plants lacking an external supply of this element. The cambium of tomato stems, for example, and the supernumerary cambiums of the storage roots of beet, sweet potato, etc., are essentially dormant. What growth occurs is therefore mainly in length and not in diameter, potassium being almost completely water soluble and freely translocated to the growing tips. A comparable response is exhibited by pineapple plants; except that, in this species, potassium when limited migrates to the relatively meristematic leaf bases and some other regions where active cell division occurs.

Carbohydrate and nitrate accumulation during the early stages of lack of potassium is associated with inability on the part of the plant to synthesize organic nitrogen from stored nitrate. This is indicated by very limited nitrate reducase activity, as determined by ECKERSON (3), and also by the fact that, when a supply of potassium is made available to potassium deficient plants, nitrite and amino acids appear and there occurs very rapid utilization of and decrease in nitrate as well as carbohydrates.

It is apparent that moderate limitation of potassium results in much the same symptoms and conditions as does a low level of nitrogen nutrition. Horticulturists and others have learned through empirical fertilizer tests to avoid excessive vegetative growth by limiting the supply of potash fertilizers. Clearly, if economically feasible, plants should be supplied with abundant potassium. Luxury consumption of potassium will not of itself produce excessive vegetative growth and depress the yield of fruit, as it is sometimes said to do. The desired quality of vegetative and reproductive growth can be much more efficiently attained by regulating the available nitrate supply.

There is considerable literature dealing with the effect of potassium on the structure of stems. Much of this is concerned with work done on grains to determine the influence of potassium upon stiffness of straw. The anatomical structure is recorded in most cases, but it is taken up entirely on the basis of the potassium treatment received by the plant, and any possible effect on the carbohydrate content is seldom considered. As might be anticipated, stiff straw is most frequently obtained, not necessarily when potassium is especially abundant, but under conditions of rather low nitrogen nutrition and carbohydrate accumulation in the plant. Potassium is frequently recorded as favoring the development of thick cell walls and stiff straw, but in many cases this element is reported as having the opposite effect. The reason is principally in the effect of a given treatment upon the rate and type of utilization of carbohydrate reserves (**8, 20**). Various workers have indicated that potassium is directly or indirectly essential for carbohydrate synthesis and hence for cell wall formation; but if nitrate is present in abundance and conditions permit rapid protein synthesis, the carbohydrate content of the plants will be low and cell walls thin regardless of the potassium present. In order to obtain cell walls of sufficient thickness and at the same time fruitful plants, the practical indication is therefore to apply enough potassium but to avoid an excess of nitrogenous fertilizers.

Under relatively extreme conditions of potassium deficiency, or following the initial stage of carbohydrate accumulation, in the practically complete absence of an external potassium supply there invariably occurs a marked decrease in content of sugars and starch. There does not seem to be failure to utilize stored carbohydrates, for HARTT's (**7**) results and those of the writer indicate that digestion of carbohydrate reserves and translocation of sugars proceed unhindered in low potassium plants. On the basis of the work of GREGORY and RICHARDS (**5**), carbohydrate depletion would seem to be correlated with an unfavorable balance between assimilation and respiration of carbon dioxide.

The later stages of potassium deficiency are therefore usually characterized by carbohydrate depletion, and with rather rapid decrease in carbohydrates there occurs a typical proteolytic response

(not in the least peculiar to potassium deficiency) yielding amino acids and related compounds. These internal changes are made manifest externally by an increase in green color of younger leaves and the development of new stem tissue that is spindling, soft, and succulent, most of the potassium in the plant migrating to these newly developed structures. Shortly following these responses the plants often die, senescence being greatly accelerated if fruits are present, since the little available potassium in the growing vegetative tissues tends eventually to be translocated to the reproductive structures.

RICHARDS and TEMPLEMAN (18) did not obtain the carbohydrate accumulation stage in potassium deficient barley plants, although they cite the results of many workers who have obtained this response with various species. They conclude, as do PHILLIPS *et al.* (17), that there is no proof that potassium bears any direct relationship to the synthesis of protein in the plant. They suggest that the accumulation of amino acids in potassium deficient plants is possibly the reason for limited nitrate reducase activity. This may well be a dominant factor under many experimental conditions, especially if opportunity for carbon dioxide assimilation is limited by the prevailing light conditions or nullified by respiration of carbon dioxide during long nights at high temperature. At least in tomato, nitrate reducase is often very low prior to, as well as following, the proteolytic formation of amino acids. Often in low potassium plants in the later stages of deficiency the percentage of amino acids and related compounds is as high or higher than in comparable plants receiving abundant potassium. But the absolute amount of organic nitrogen is of course very low, as potassium deficient plants are relatively small.

Obviously the function of potassium is little understood and any effects recorded may well be indirect. Nevertheless in practical plant nutrition it is important to avoid a deficiency of either organic nitrogen or carbohydrates, and potassium directly or indirectly plays an important part in determining the amount and concentration of these materials found in the plant.

CALCIUM DEFICIENCY.—Although the respective initial symptoms of lack of nitrogen, magnesium, phosphorus, and potassium do not

usually appear simultaneously in a given series of plants, the effect upon their external appearance, especially during early stages, is often very similar but may vary materially with the character of the nutrient or soil solution and opportunity for carbon dioxide assimilation. In tomato and in apple (1, 13, 14) grown in sand culture, in all four cases of nutrient deficiency the older leaves were typically yellowish green and the younger leaves and tip of the stem were fairly dark green and usually remained so for a considerable period.

On the other hand, the appearance of calcium deficient tomato plants and apple trees was very distinctive, in that the upper part of the growing tips of the tomato plant and the distal portion of the current twig and leaf growth of apple trees was yellowish, as a rule, whereas the first formed leaves remained relatively dark green even after the stem tip was dead or all elongation had ceased. These results are not peculiar to the species mentioned, for almost identical responses have been recorded for other kinds of plants (4). Boron deficiency is also associated with injury to young rather than to mature tissues (2).

The root systems of calcium deficient plants were drastically affected. Frequently the roots practically failed to develop and those that did appear were characteristically short, bulbous, and brown at the tips, with sloughing off of cells farther back. The roots were short because of a slow growth of the meristem, and bulbous because cortical cells enlarged somewhat more laterally than longitudinally. The failure of lateral root primordia to develop sufficiently to emerge through the cortex also contributed to the bulbous appearance.

When new cells are formed, there must not only be calcium presumably for development of the middle lamella, but also available calcium for combination with materials of the protoplast. When calcium was deficient, granular proteinaceous inclusions accumulated and there was only limited absorption and reduction of nitrate.

Plants possess in a considerable degree the capacity to re-utilize contained nitrogen, magnesium, phosphorus, and potassium. Each of these elements when limited tends to migrate from mature to relatively meristematic zones of roots and tops. However, as PETRIE'S (16) work indicates, calcium is much less mobile in the plant than

the elements mentioned; it accumulates in the leaves but it does not freely migrate from them again, so that the supplies for each successively formed structure must be derived very largely from the nutrient or soil solution.

This accumulation of immobile calcium occurred in tomato and apple not only in the comparatively dark green lower leaves but in the older portions of stem tissue and roots. Calcium was stored in the form of calcium oxalate and also in combination with proteins or other materials, as "combined calcium." Although tomato plants which received an external supply of this element contained considerable amounts of mobile, water soluble calcium, nearly 100 per cent of the calcium of the fresh tissue of the calcium deficient plants was water insoluble and most of it was located in the older tissues of roots and tops. Nevertheless the utilization of calcium oxalate and the re-utilization of combined calcium took place so slowly that root and stem tips died while there were yet heavy deposits of calcium oxalate and a high concentration of combined calcium in the older tissues.

The nature of combined calcium has not been determined, but it should be emphasized that it is released and made available to meristematic tissues, and new growth occurs following any treatment, such as continuous darkness, shading, or pruning. Calcium deficient plants are often very high in carbohydrate reserves, but carbohydrates can readily be decreased on shifting such plants to darkness or by defoliation or pruning. Accompanying digestion and decrease in stored carbohydrates there is a typical proteolytic response, release of combined calcium and translocation of the resulting soluble calcium to the developing organs, along with the hydrolytic products of protein and carbohydrate reserves.

This response in calcium deficient plants on decrease in carbohydrates and likewise on shift from minus to plus calcium nutrient treatment is apparently similar in principle and effect in certain respects. In both cases soluble mobile calcium is made available for combination with newly formed proteins or other materials. A supply of amino acids for formation of new proteins of meristems is likewise available in both instances in the low carbohydrate,

calcium deficient plants through proteolysis and in the plants newly supplied with calcium through nitrate absorption and assimilation.

Certain responses associated with calcium deficiency have been recorded, but it must be admitted that little is known concerning its function. Lime is commonly used to neutralize excessive soil acidity, but non-acid soils are sometimes deficient in calcium. Often during a period of drought the calcium content of certain soil solutions apparently becomes very low. At least the roots of fruit trees in the field exhibited all the external symptoms of a deficiency of this element and contained little or no uncombined calcium. Especially in the case of apple and peach trees, a deficiency of calcium may be very drastic, owing to its effect on the fine rootlets, which are the organs chiefly concerned in the initial phases of protein synthesis in the fruit trees mentioned (**14, 15**).

PINEAPPLE EXPERIMENT STATION
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HISTOLOGICAL REACTIONS OF BEAN PLANTS TO GROWTH PROMOTING SUBSTANCES

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(WITH FIFTY-THREE FIGURES)

Introduction

In previous papers (**2, 5**) some of the histological responses of the bean and of the tomato to applications of indoleacetic acid in lanolin were described. Studies on the bean, which responds readily to wounding (**6**) and environmental changes, and other plants have been continued and further details with reference to the former are presented herewith. In general the same techniques have been followed, except that in addition to terminal application of lanolin mixture (30 mg. indoleacetic acid per gram of lanolin) to decapitated stems, the same mixture has been applied as a narrow band completely encircling the uninjured internodes of stems, to the slightly abraded surfaces of young pods along the adaxial suture, and over the cut terminal surfaces of similar pods. Also two additional substances, indolebutyric acid and naphthaleneacetic acid, both received through the courtesy of DR. P. W. ZIMMERMAN of the Boyce Thompson Institute, have been used in mixture with lanolin.

Investigation

OLD TERMINAL TUMORS RESULTING FROM APPLICATION OF 3 PER CENT INDOLEACETIC LANOLIN MIXTURE

A report has been made (**5**) upon the developmental stages of apical tumors resulting from application of a 3 per cent mixture of indoleacetic acid in lanolin up to a period of 168 hours following application (fig. 1). Such tumors may continue to grow and develop for periods longer than six months, frequently attaining a diameter

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of 2 cm. and more and a total height above the original cut surface of the stem of 2–2.5 cm. (figs. 2, 3). The surface of such old tumors is tuberculated or warty, each tubercle or wart generally tipped with

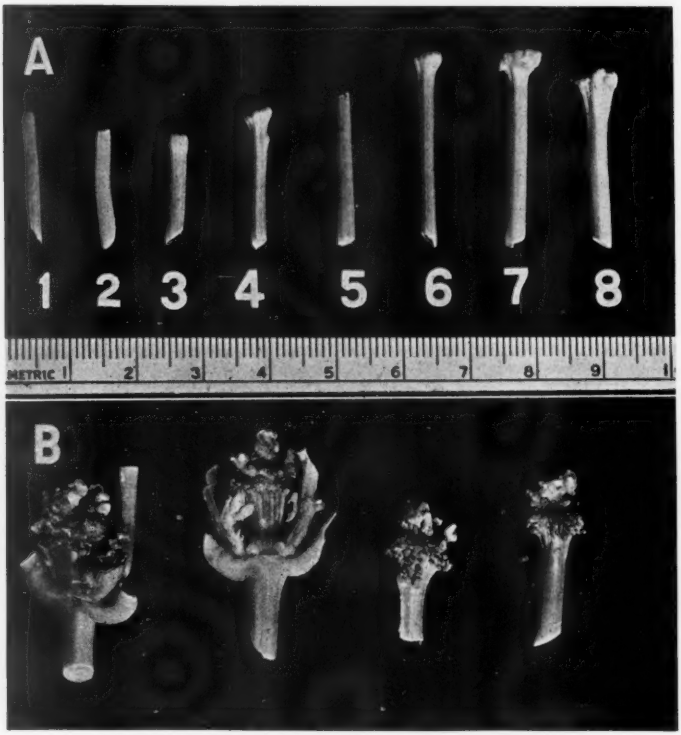

FIG. 1.—Stages in tumor development (time given in hours after treatment with 3% indoleacetic acid lanolin mixture). *A:* 1, just after decapitation; 2, 30 hours; 3, 48 hours; 4, 66 hours; 5, 72 hours not treated; 6, 72 hours treated; 7, 144 hours; 8, 168 hours. *B:* four apical tumors 28 days after decapitation and treatment of stem. Adventitious roots have not emerged, but prominent ridges and rows of small tumors over vascular bundles are evident, as is also central apical mass of proliferating tissue. Natural size.

a bit of lanolin mixture which apparently slowly supplies the indoleacetic acid which acts as a stimulus for continued development. As development proceeds the crown of roots and root primordia be-

comes more or less completely imbedded by the expanding tissues. If the surrounding atmosphere remains relatively dry, little development is made by these roots and frequently many of them die or partially disintegrate; but if the surrounding atmosphere is saturated with moisture, or if wet sand or other moist medium is placed in contact with the zone of roots, they develop actively, grow out for an indefinite length from the stem or tumor, and produce secondary roots in abundance.

Microscopic inspection of the old tumor above the root zone shows that it was developed largely from proliferated tissue of the pith (figs. 3, 4), although parenchymatous cells of the phloem and xylem which also remained meristematic gave rise to derivatives which contributed to its bulk. As the tumor progressively enlarges and the lanolin mixture becomes somewhat dry and toughened, this covering mixture becomes fragmented into irregular bits or small masses. Directly beneath such a mass the cells proliferate, expand, and differentiate more rapidly, with the result that the surface becomes more and more tuberculated and the separated small masses spread farther apart. There is, however, decided activity on the part of many of the cells at the base of and between the tubercles, so that a considerable volume of tumor is also built up between them. Some derived cells differentiate into strands of xylem, phloem, or both, with zones of active cells between and around them, so that the whole structure becomes highly vascular, anastomosing strands and patches of xylem, phloem, and parenchymatous cells occurring in every conceivable relation to one another. Many of the parenchymatous cells remain active and more or less continuously give rise to derivatives, some of which in turn differentiate into tracheids, sieve tubes, companion cells, or other vascular elements. At the periphery of a tumor most of the cells become suberized, but directly underneath these others divide actively and from them vascular elements are differentiated. In observing a cross section through such a developing tumor, one is strongly reminded of a similar section through a fleshy root of a sweet potato or a radish the secondary xylem parenchyma of which has undergone or is undergoing tertiary thickening.

As previously stated, some of the adventitious roots may die;

others may become much compressed or imbedded owing to the activity of the phloem cells and phloem derivatives (figs. 4, 5). Slightly below the root zone, the stem may enlarge but little, and secondary thickening proceed in the usual manner. Centrifugal to the secondary phloem of many of the principal vascular bundles vascular strands originally derived from endodermal or primary phloem cells may continue to develop, thus approaching in general appearance the original stelar bundles. Such vascular strands frequently extend more than 10 cm. below the original treated surface (figs. 5, 6), although they rarely exceed 6 or 8 cm. in length. From the parenchymatous tissues which partially compose these outermost bundles root primordia may be initiated, and if ample moisture is provided, continue development for an indefinite period. In other respects the stems at 1–10 cm. below the apical tumor appear as do stems which have not been treated and have undergone the usual type of secondary thickening.

TUMORS RESULTING FROM LATERAL APPLICATION OF 3 PER CENT INDOLEACETIC ACID LANOLIN MIXTURE TO SECOND INTERNODE

The lateral applications to the second internode of the stem were made on plants the same age as those receiving the terminal applications at the time the first trifoliate leaf was beginning to spread out. The same strength of mixture was employed. By the use of a small pointed scalpel the mixture was drawn out into a thin thread, and this thread was then carefully laid on the stem so as completely to encircle it. Great care was used to avoid any possible mechanical injury to the surface of the stem, and through the method employed this was easily accomplished. In a few instances the line of application was marked with India ink, but this was needless, and in fact when the ink lay directly under the mixture, reaction of the stem at that point was frequently delayed.

Within 30 hours after application the internodes showed decided bending in one direction or another, but usually straightened out within 48 to 72 hours. The trifoliate leaf was bent downward at first but soon assumed its original erect or horizontal position. Marked yellowing and swelling of the stem occurred 2 or 3 mm.

above, below, and at the line of application. Such effects became more marked during several successive days, until by the end of a week or ten days the tumors were often 2 cm. or more long, spindle shaped, and markedly ridged over the vascular bundles. The line of mixture became stretched both vertically and in circumference to a band 2 to 3 mm. wide. Through this band and also above and below it, roots emerged, occurring in longitudinal rows of five to fifteen or more, mainly between the swellings over the vascular bundles. Under average greenhouse conditions these rarely emerged more than 2 to 3 mm. beyond the surface, but would grow apparently indefinitely long in saturated atmosphere. In nearly every case the swelling and the number of root primordia initiated both above and below the line of application were about equal (fig. 13). In no case did swelling or root differentiation occur as far below this line as was the case when the mixture was applied to the cut surface of decapitated stems. Very different results occur when the plants are grown continuously in the dark, but such results will be presented at another time.

If the atmosphere was not saturated with moisture the tumors apparently ceased active expansion in ten days to two weeks and became dull brown in color instead of light yellow; the surface cracked slightly. Almost without exception, in many different experimental lots and at different seasons, this quiescent stage extended over a period of about two weeks. Some of the tumors never grew further, although the cells composing them remained alive, but most of them again became active. The longitudinal cracks then became wider and the tumors grew rapidly. Winglike projections developed either from one side of the tumor or about its whole periphery. On their surfaces these wings carried out bits of the lanolin mixture. The rate of growth at various places became still more irregular, resulting in a rough tuberculated contour closely resembling that of apical tumors. At times growth was rapid, again slow; often a sort of rhythmic development took place. By the end of four to six months these lateral tumors measured 1–4 cm. in greatest diameter, and were still actively developing (fig. 9).

The presence of these tumors brought about little or no retardation of the growth of the stems, either axial elongation or expansion

in diameter, and the plants fruited as well and as abundantly as plants which had not been treated (figs. 7, 8).

The histological response of uninjured stems treated laterally closely resembled those of stems decapitated and treated terminally, although there are some striking differences. Within 30 hours after treatment, the cortical cells of laterally treated stems had elongated radially and by 48 hours the cells of the endodermis had begun division (fig. 11). Within 72 hours there was marked meristematic activity of some of the inner cortical parenchyma, the endodermal derivatives, the primary phloem, the cambium, and the cells of the rays flanking the phloem. The ray cells adjacent to the xylem and the pith enlarged somewhat but showed no pronounced meristematic activity at any time. This behavior of the pith is in sharp contrast to that of stems decapitated and treated apically. By 96 hours the root primordia were clearly defined in the outer portion of the rays. The phloem parenchyma proliferated markedly, as did also the cambial derivatives. Many of the outer cortical cells became stretched and torn; in some instances vascular strands were initiated from endodermal and phloem derivatives. The pericyclic cells showed some meristematic activity but little progress toward maturation as fibers.

By the ninth day (216 hours) the root primordia were well differentiated and may or may not have pushed through the surface; the derivatives of the cambium matured either as elements of the phloem or xylem; but there was little or no activity of the ray cells adjacent to the primary xylem, nor did the pith cells become meristematic. Centripetal to the bases of the roots and the inner portions of the primary phloem the whole stem appeared much like one of the same age which had received no treatment with mixture, except that the cambium was less well defined and the secondary xylem thinner walled (fig. 14*A*).

As previously stated, at about this time marked cell proliferation became greatly diminished and the whole stem showed relatively little change except for thickening of cell walls, some additions to the secondary xylem and phloem, and the number of phloem cells between the adventitious roots. On the twenty-third day it appeared much the same as on the ninth day (fig. 14*B*). In fact, further

marked changes did not occur for weeks or months in some plants, but in others the proliferated parenchymatous cells of the primary phloem and of the outer portions of the secondary phloem became more or less abruptly highly meristematic. Some of the derivatives matured as tracheids, others as vascular elements. Both xylem and phloem differentiated in every conceivable arrangement and condition of anastomosis, with highly active embryonic cells scattered among and exterior to these vascular strands and masses. Thus a mechanism becomes established whereby there may be continued cell proliferation, and from such derivatives vascular elements may continue to differentiate apparently indefinitely (fig. 15*A*, *B*). The adventitious roots became imbedded by these surrounding flanges and tubercles of proliferating tissue, although they were readily stimulated to further development if the tumors were surrounded by moist atmosphere or other moist medium. Under the latter conditions they pushed out through the surrounding masses of tissue and grew to indefinite length. Among the number of old tumors sectioned up to five months after treatment, the pith cells never have shown conspicuous activity. Rather they appeared much as they would in an untreated stem. Sometimes they contained a few starch grains near the xylem and were often dead, torn, and more or less disintegrated at the center of the stem (fig. 17).

Apical tumors resulting from application of indolebutyric acid lanolin mixture

For these experiments the plants were grown, decapitated, and treated just as were those plants used in the experiments on tumors resulting from indoleacetic lanolin mixture. The mixture was made up by adding 15 mg. crystalline indolebutyric acid to 1 gm. pure anhydrous lanolin at its melting point, stirring the two together thoroughly until a homogeneous mixture was obtained, and continuing such stirring and mixing until the whole mass solidified, thus avoiding the possibility of settling out of the indolebutyric acid.

In general the responses of the stems to this mixture are similar to those to 3 per cent indoleacetic acid lanolin mixture, with certain conspicuous exceptions. Within 48 hours the cortical cells showed marked enlargement, the endodermis and many cortical cells

adjacent to it proliferated, the primary phloem cells became active, and a few of the ray cells became meristematic near the cambium. By 72 hours the endodermal cells had proliferated very greatly, apparently much more actively in some areas than others, especially over the larger vascular bundles; the phloem was very active; in places the beginning of vascular strands from the phloem derivatives was evident. The rays were active, but there was only slight activity of the pith and that adjacent to the xylem vessels. By 112 hours the young roots were well defined, more frequently opposite a ray (fig. 20) but often over the phloem of one of the larger vascular strands (fig. 22). The pith cells had become highly meristematic and from them vascular strands and masses were differentiated; others continued actively meristematic and from their derivatives the process was repeated over and over. Thus through a period of time large apical tumors were developed, closely resembling those resulting from applications of indoleacetic acid lanolin mixtures.

A striking characteristic resulting from apical treatment of stems with indolebutyric acid is the broad heavy roots which are produced, and the fact that many of these develop over the phloem instead of from the rays; or often they may be very broad and involve not only the mass of cells in the phloem of a vascular bundle but also parts or all of the two adjacent rays. Such roots first begin in the endodermal cells over the phloem (fig. 21*B*) and gradually the cells of the phloem itself become involved. The xylem of such adventitious roots usually differentiates from derived cells of the rays adjacent to the phloem, and thus a connection with the xylem elements of the vascular bundle is effected. In many instances the phloem derivatives or endodermal derivatives themselves differentiate as vascular strands over the phloem of the vascular bundle, and the vascular system of the young root which lies centrifugal to them differentiates in such a manner that its conductive system is directly connected with a vascular strand or strands which lie centripetal to the vascular bundles of the main stem. Roots formed in this way frequently die at an early age, although under appropriate environmental conditions, they may continue to grow and develop as do those derived from the rays and endodermis (fig. 23).

The effects of the indolebutyric acid seem to be manifested at greater distances down from the surface of application than do those from indoleacetic acid, and very frequently the adventitious roots occur in three or four tiers close together near the cut surface, and a few scattered ones may appear in a circle 5 to 10 mm. below the surface of application (fig. 25).

Apical tumors resulting from application of naphthaleneacetic acid lanolin mixture

The naphthaleneacetic acid lanolin mixture was prepared in the same manner as usual, except that only 5 mg. of the substance to 1 gm. of lanolin was used. The plants employed and methods of application did not differ from those in other experiments. The main gross and histological effects resulting from application of this mixture are similar to those of indoleacetic or indolebutyric acid. There are two obvious differences. First is the far lesser tendency to produce a vascular apical tumor above the surface of application, the tumors usually being relatively wide and flat topped with slightly scaly but not tuberculated surfaces. The second striking difference is the marked swelling of the tissues over the principal vascular bundles for a distance of 1 cm. or so below the treated surface, this swelling then terminating abruptly, so that a distinct ring of bumps or a conspicuous shoulder is formed. At each of these bumps one to several large root primordia are developed. Below these points swelling is much less but is still obvious to 8–10 cm. below the cut surface (fig. 30).

Histologically there are also some striking differences. The cortical cells, even those immediately below the epidermis, proliferate markedly. The endodermal cells are extremely active; the pericyclic cells proliferate but slightly, but the phloem, ray cells, and cambium derivatives become highly meristematic. The pith cells show much less response. By the end of 48 hours the pith cells next the protoxylem and metaxylem had dense content and showed a few divisions, and by the end of 72 hours there was a slight increase in the number of such derivatives, especially close to the surface of application, but 1–2 mm. from it there was little proliferation. By the end of 96 hours root primordia were evident in the rays; the phloem

and cambium derivatives had greatly increased, but generally there was no continued increase in activity of the cortical or endodermal cells. At the end of 120 hours after application, the young roots were clearly outlined, almost exclusively derived from the rays. The pith cells showed little activity except immediately adjacent to the primary xylem vessels, although at 215 hours the pith was markedly meristematic at a distance of one or two layers of cells beneath the surface of application. From such derivatives of the pith vascular masses were differentiated (fig. 29). Just below this zone the pith cell showed only occasional divisions.

As has been stated, the endodermal cells are generally extremely meristematic. From their derivatives over each vascular bundle large vascular strands differentiate and these are progressively enlarged from the cambium-like cells between their xylem and phloem. At the periphery of these strands the endodermal cells continue to proliferate actively and give rise to additional strands farther and farther away from the center of the stem. Some of these large masses of endodermal derivatives, however, lying a centimeter or so below the treated surface, together with the derivatives of two or more adjacent rays, may give rise to a single huge root primordium or to several primordia so close to one another that they appear more or less fasciated (fig. 30). These are the conspicuous bumps referred to in the gross description of these tumors.

As previously stated, activity of the endodermis and phloem parenchyma may extend for considerable distances down the stem, but the pith seems never to become as active as it does in tumors resulting from applications of indoleacetic acid or indolebutyric acid mixtures. Rather its cells mature, generally die at the center of the stem, and finally become disrupted and disintegrated.

Comparison of tumors resulting from the three growth substances using equivalent concentrations

As most of the experiments were carried out with indoleacetic acid (30 mg. per gram of lanolin), indolebutyric acid (15 mg. per gram), and naphthaleneacetic acid (5 mg. per gram), the question arose as to whether the characteristic differences shown by the tumors would

persist when all three substances were used at the same concentration. To test this, each substance was mixed with lanolin at the rate of 15 mg. per gram. All plants used were as nearly uniform as possible, and all applications were made at the same time. The results showed that the characteristic differences did persist (fig. 32). The higher percentage of naphthaleneacetic acid proved somewhat more toxic than the lower, with the result that the cells for several layers below the surface of application were killed (fig. 33), but beneath these the reactions were characteristic. Three weeks following application, the tumors resulting from application of the 1.5 per cent indoleacetic mixture were practically identical with those from the 3 per cent mixture; and those from the stronger concentration of naphthaleneacetic acid showed no more tendency toward proliferation of the cells of the pith and the building of a large apical vascular tumor than did those resulting from the use of the lower concentration (fig. 34).

Tumors resulting from application of 3 per cent indoleacetic acid mixture to pods

As shown previously (**5**), the pod of the bean responds actively to application of indoleacetic acid in lanolin. Bean pods have long been used as experimental material in testing growth substances (**7, 4**). We decided, therefore, to extend our histological studies to them. In all cases here reported a 3 per cent indoleacetic acid lanolin mixture was used. At first pods of various sizes and ages were used, and all responded; but it was finally decided to study those most critically which had attained a length of approximately 5 to 7 cm. and a suture to suture diameter of approximately 5 to 7 mm. (fig. 37*B*).

Two types of application were made. In one the pod was very lightly rubbed with a piece of fine emery cloth along the adaxial suture in order to remove the hairs from the surface and just slightly abrade the epidermal cells (fig. 44*A*). The mixture was then spread thinly and evenly along the suture, great care being taken to cause no further mechanical injury to the pods. Response was prompt and decisive (fig. 36). Pods not brushed with emery cloth respond to applications of the mixture also, but such response is far less uniform throughout their length unless the mixture is pressed down firmly

with the spatula to make sure that it comes evenly in contact with the epidermal cells. The other method of application consisted of cutting off the pod squarely with a sharp knife about one-fourth its length back from the tip, blotting off the exudate with lens paper, and then applying evenly over the cut surface a thin film of the mixture. Care is required in doing this to avoid crowding the mixture into the cavity of the pod, thus bringing about an uneven distribution over the exposed surface.

The gross responses of pods may differ appreciably in degree. In general larger and more extensive tumors are produced on pods during periods of bright rather than dull weather, and on the vigorous and vegetative plants rather than on those which are spindling or old and making little growth. Pods to which the mixture has been applied along the adaxial suture frequently develop no seeds, although they may do so if the mixture is applied after the seeds are about halfgrown. Those which had the mixture applied to the cut end produced seeds in at least half the cases in our experiments. In general if the seeds continue to develop the apical tumor is smaller than on those pods in which the seeds fail to continue development (fig. 35). Generally also, if a pair of pods is selected, one of them treated and the other not treated, the treated one will remain more or less succulent and green over a longer period than will the untreated. Several apically treated pods produced tumors and roots which, lying in contact with moist soil, continued to develop. These rooted pods remained green for nearly four months, finally dried and dehisced. The seeds fell from them, although the tumors at their tips remained green and continued to enlarge for more than two months longer, when they finally ceased to grow and decayed.

Figure 37 illustrates three stages in the development of the pod and seed of the Red Kidney bean. The transverse section shown at *A* is from a fruit shortly after fertilization has taken place. The petals have withered and the pod has begun enlargement. The adaxial suture is at the right, the abaxial at the left. For convenient reference the main body of the pod may be divided into an endocarp, indicated by the two or three innermost rows of blocklike cells, a mesocarp several cell layers thick and including the entire system of vascular bundles with parenchymatous cells between them, and

an exocarp several cell layers wide and without vascular strands. At *B* a similar pod several days older is shown. This is the stage of development of the pods when the mixture was applied to them. The three zones are even more clearly delimited, especially the endocarp which is several cell layers thick. The endocarp is apparently a much proliferated epidermis entirely without vascular elements and in the fresh pod is of a translucent or slightly water soaked appearance. *C* represents a transverse section through a nearly mature pod and seed. The endocarp has lost its succulent character and has shrunken against the mesocarp. The cells of the exocarp have also dried out and shrunken appreciably. Such a pod under average greenhouse conditions would become dry and dehisce within four to six days subsequently.

TERMINAL APPLICATION.—While there is some range in the time and degree of response of pods on different plants when grown under average greenhouse conditions, this is not great. Within 30 hours after application the parenchymatous cells of the exocarp and mesocarp adjacent to the treated surface have become yellowish, enlarged appreciably, and frequently a few divisions in various planes have taken place. By the end of 48 hours the number of such divisions has increased markedly, and a few of the cells of the endocarp may also show divisions, although these are uniformly slower in response than the cells of the other two regions. By the end of 96 hours (fig. 38) the parenchymatous cells of the exocarp and mesocarp have proliferated markedly and some of the derivatives of each region have begun to differentiate as vascular elements, so that the clear line of demarcation between the two regions is more or less obliterated. The cells of the mesocarpic portion are smaller than those of the exocarp. The cells of the endocarp near the surface of application also have begun to proliferate, but lag far behind those of the other two regions, with the result that the edges of the pod protrude markedly beyond the central portion. The endocarp remains completely non-vascular. Stages five and six days after application showed progressive development of the two outer regions, with marked increase in vascularity of the proliferated portions, and in many specimens a great increase in activity of the cells of the mesocarp also. By the end of twelve days the two outer portions

had so greatly proliferated that they not only flared markedly outward but also inward and over the endocarp, their edges close together. The whole apical tumor as seen externally consisted of proliferated cells derived from the exocarp and mesocarp (fig. 41*A*). During this same period primordia of roots, derived mainly from the derivatives of cells of the exocarp (fig. 41) but also from those of the mesocarp, differentiated. These generally protrude from the surface of the tumors. Such proliferation of the cells of all the regions may continue for several weeks, with increasing vascularity of the tissues derived from the exocarp and mesocarp; but none of the cultures showed any differentiation of vascular structures in the endocarp at any stage of its development. This is of some interest in that BONNER (1) has stated that similar material from the pod of the bean grown by him in tissue culture continued to grow and develop as parenchymatous tissue, and there was no differentiation of it into root and shoot nor into vascular tissue.

The cells of the exocarp and mesocarp do not respond to any great extent below the surface of application of the mixture. Apparently the indoleacetic acid is conducted but slightly through either the phloem or xylem. This effect is in contrast to the condition prevailing in the stem. That the cells near the surface of the endocarp show an apparent response at appreciable distances from the cut surface may result from the spreading of the mixture, or of some of the indoleacetic acid which may diffuse from it, over the surface of the endocarp which becomes moistened by the exudate, which is often copious at the time of cutting or for a considerable period thereafter. In fact, the response of the endocarpic cells to the treatment at some distance from the cut surface may be more apparent than real, for these cells show definite proliferation in pods that have been only cut and not treated, or in those left to develop with no treatment whatsoever.

APPLICATION ALONG ADAXIAL SUTURE.—After the mixture had been applied along the adaxial suture, the cells of the exocarp near the surface of application became meristematic, the divisions occurring at first mainly tangentially, then in all directions. By the end of the third day after application, some of the derivatives of these cells began to mature as vascular elements in scattered groups or

FIG. 2.—Apical tumor 31 days after treatment with 3% indoleacetic lanolin mixture. Nearly all vascular mass above level of application indicated by upper portion of zone of roots has been derived from the pith. Activity has continued to extend down stem also, with consequent differentiation of root primordia at considerably lower levels.

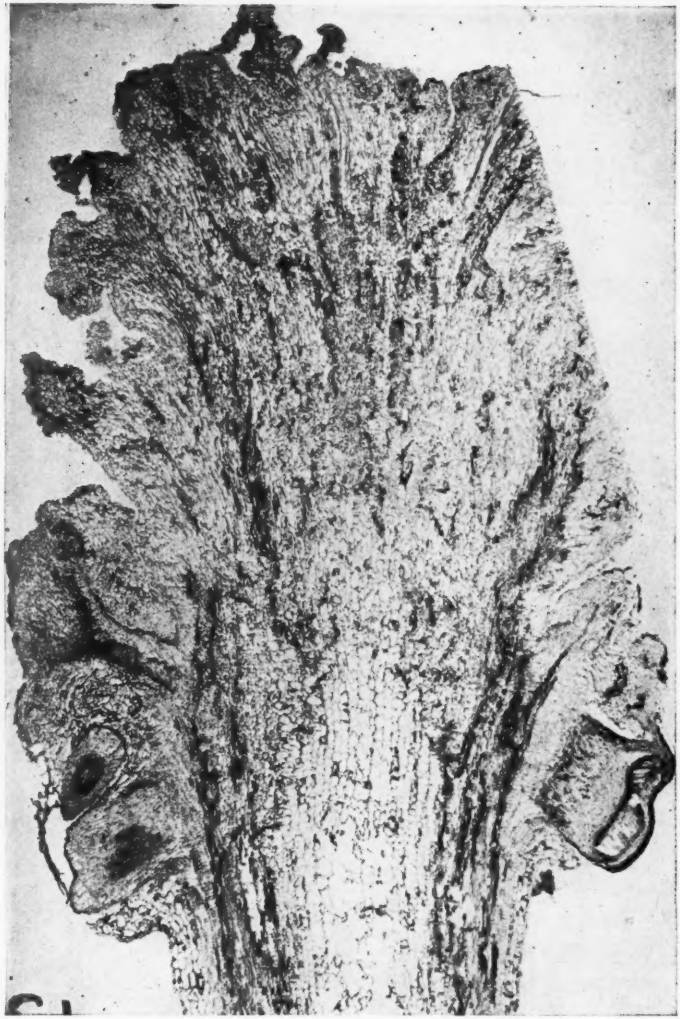

FIG. 3.—Same type of tumor 41 days after application of lanolin mixture. Most of vascular mass above surface of application is from the pith, that among and overtopping adventitious roots is mainly from the phloem. Pith shows very slightly increased meristematic activity or vascularity a short distance below original cut surface. Tubercles at top and periphery of tumor were still tipped with some of originally applied lanolin mixture and growing actively. Details of this activity were of same pattern as shown in figs. 50–53.

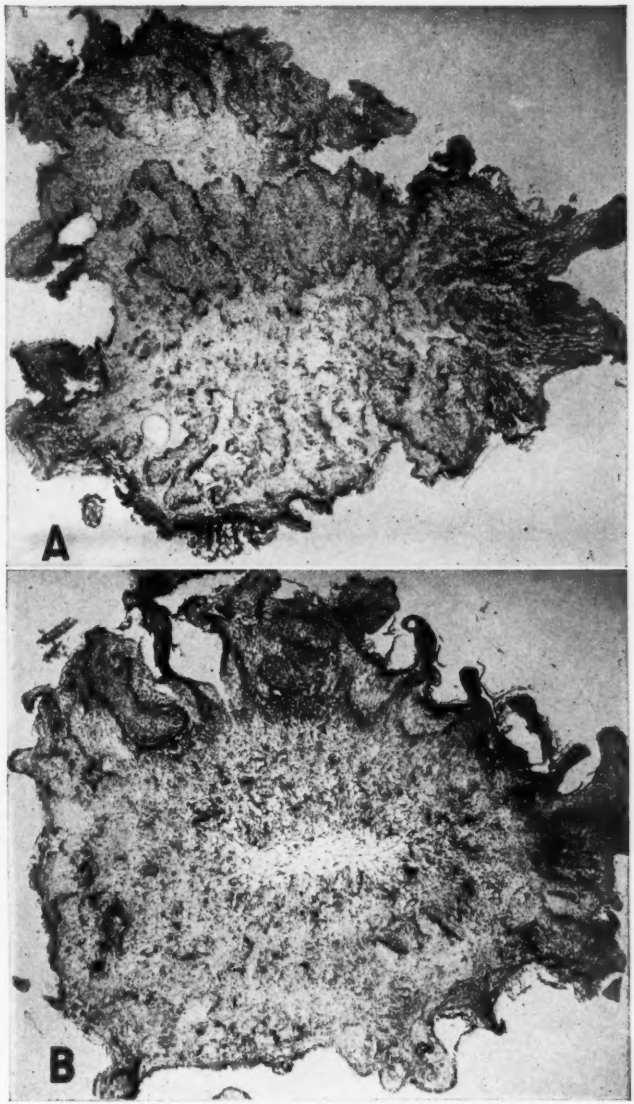

FIG. 4.—Transection of old tumor 77 days after application of lanolin mixture, and still actively developing. *A:* through tumor developed above surface of application; meristematic activity, especially at periphery, very great. All tissues from the pith. *B:* through upper level of root zone just below surface of application. Many root tips have died and been overgrown by derivatives of phloem. Pith, especially at periphery, is meristematic, but not so much so near the center.

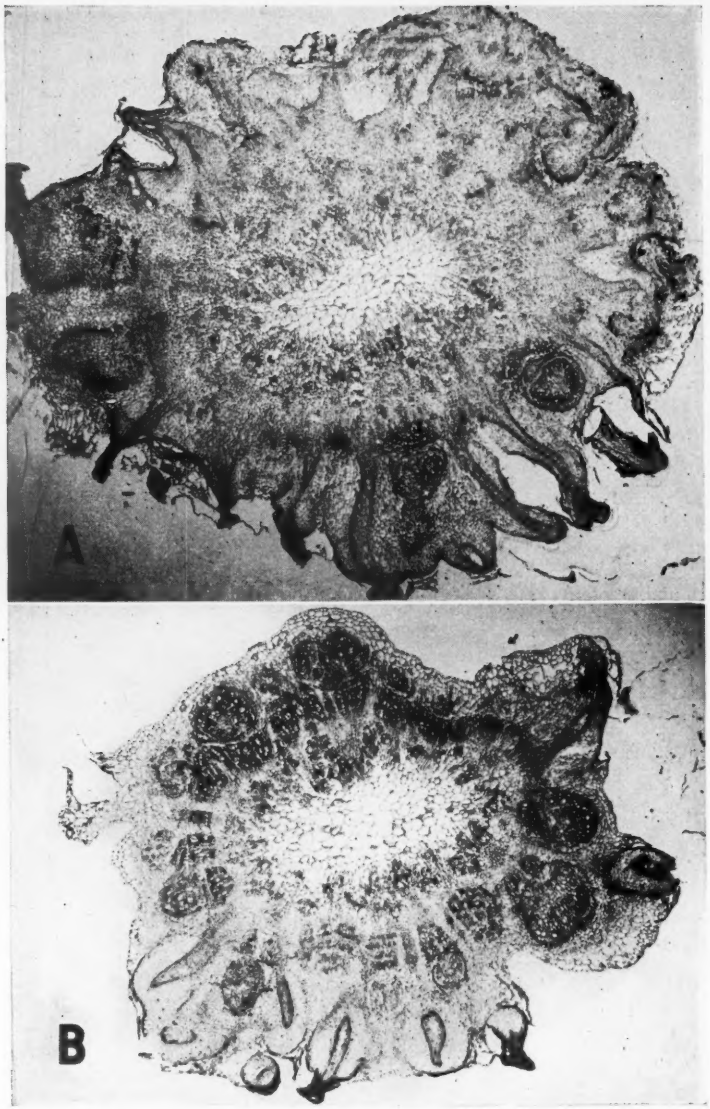

FIG. 5.—Same tumor as fig. 4. *A:* section at base of root zone. The derivatives from the phloem, the large, compact vascular masses derived either from the endodermis or phloem lie between many roots, some of which, especially their bases, are still living. Pith at center has shown little proliferation. *B:* same tumor about 1 cm. below treated surface. Bases of roots, mainly related to rays, still living. Large vascular masses over main vascular bundles are being added to by a cambium between their xylem and phloem, and frequently smaller bundles still outside these are differentiated from parenchymatous cells derived from inner cortex or endodermis.

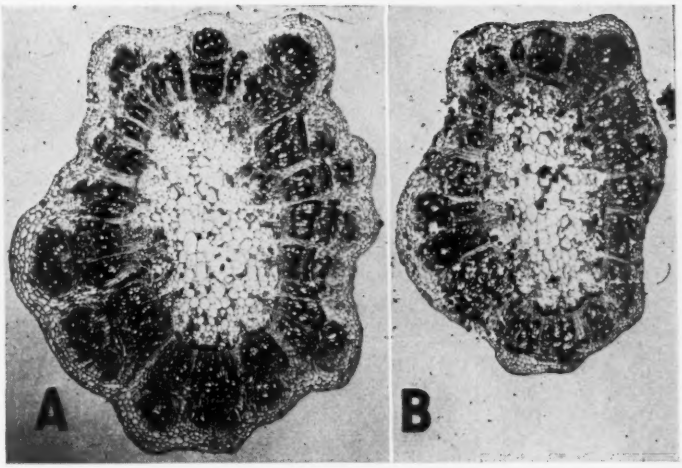

FIG. 6.—Same tumor. *A:* 3 cm. and *B:* 5 cm. below treated surface. Vascular strands derived from endodermis or phloem lie centrifugally to main bundles of stem which have undergone usual type of secondary thickening. Pith shows little or no meristematic activity. Same scale as figs. 4, 5.

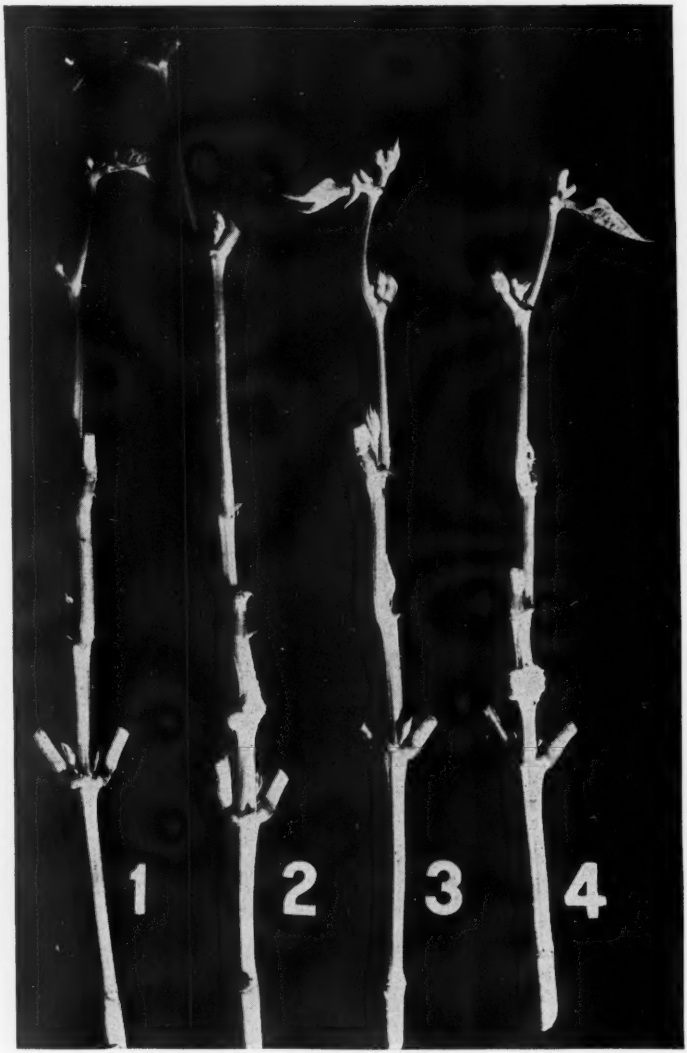

FIG. 7.—Stems 2 and 4 treated laterally with a ring of 3% indoleacetic acid lanolin mixture; 1 and 3 untreated. All 13 days after treatment. Apart from formation of the tumors and adventitious roots on treated stems, the two types were identical, with large green leaves and beginnings of flowering branches.

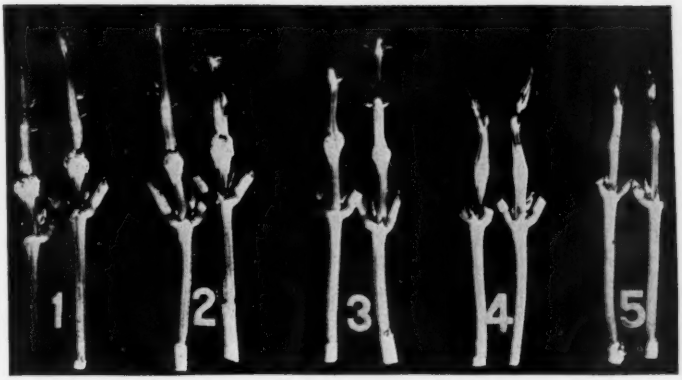

FIG. 8.—Lateral treatments around the stem, 1, thirteen days; 2, nine days; 3, six days; 4, three days; and 5, one day after application, showing rate of development of tumor. Natural size.

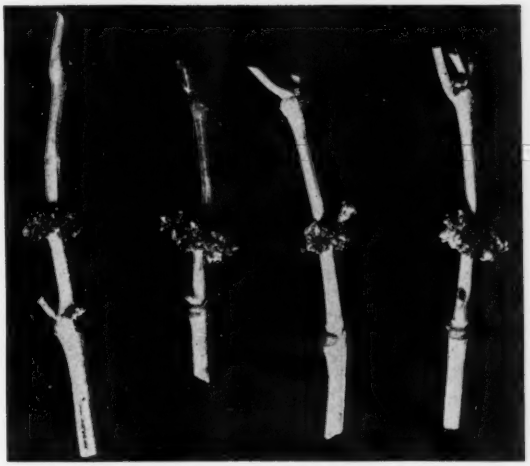

FIG. 9.—Old tumor resulting from lateral treatment 160 days after application of lanolin mixture. Tumors still green and growing slightly, although plants as a whole were greatly lacking in vigor, each having produced many flowers and seeds. Reduced about one-third.

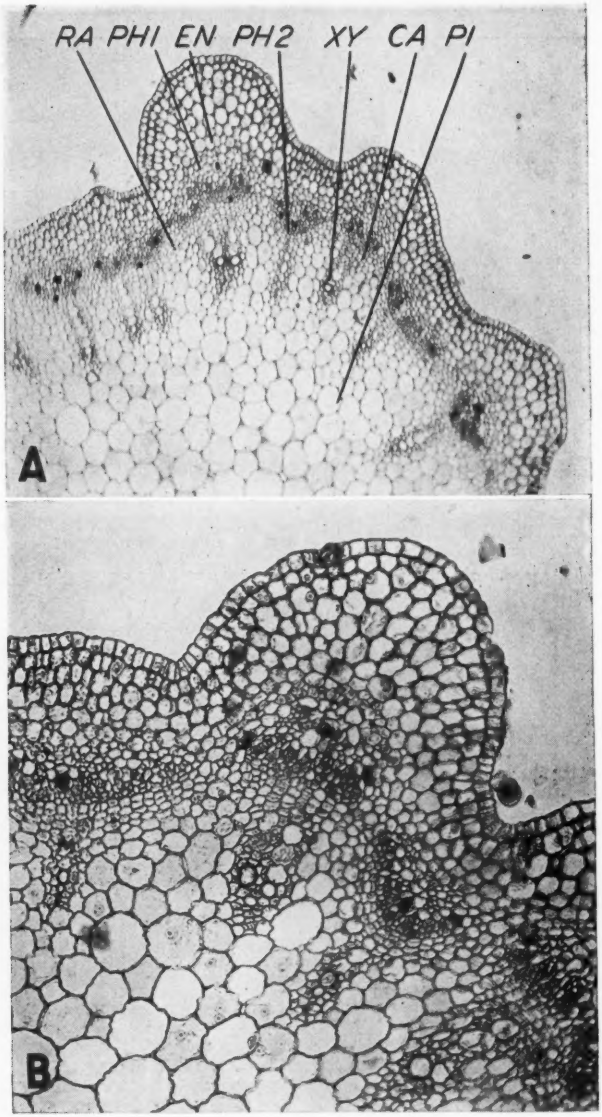

FIG. 10.—*A:* transection near top of second internode of bean at time of initial treatments, showing various tissues and stage of development (*ra*, ray; ph_1, primary phloem; *en*, endodermis; ph_2, secondary phloem; *xy*, xylem; *ca*, cambium; *pi*, pith). *B:* sector of same enlarged; pericyclic fibers over smaller bundles more nearly mature than those over the larger.

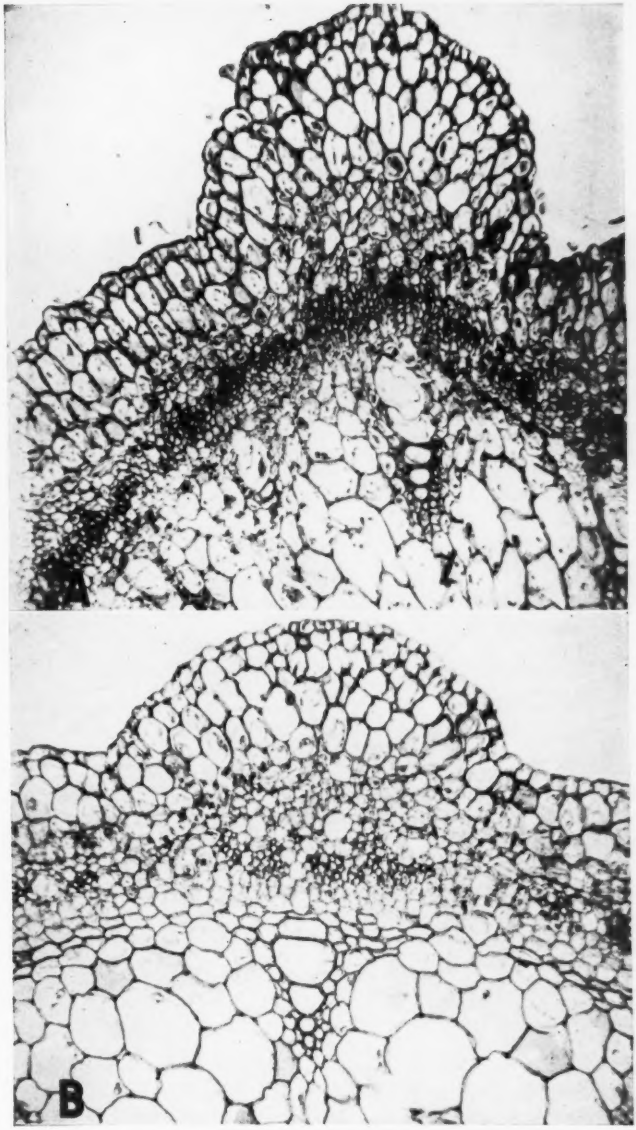

FIG. 11.—*A:* 30 hours and *B:* 48 hours after lateral treatment; sections midway of applied band of lanolin mixture. In former the cortical cells have enlarged markedly, particularly radially; in latter the endodermal cells show first tangential divisions.

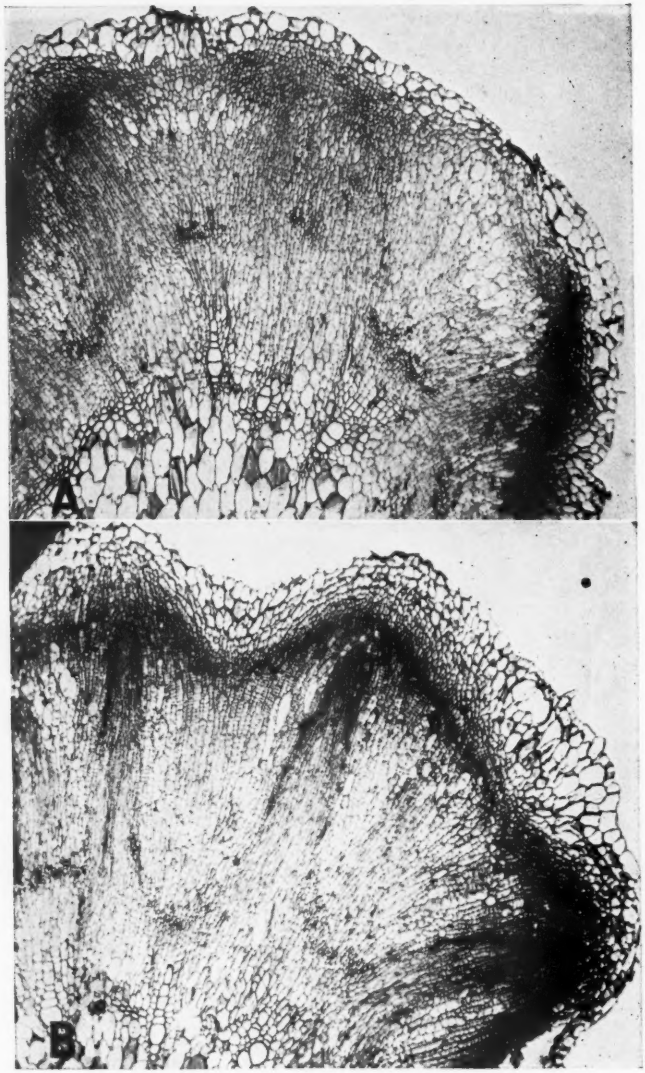

FIG. 12.—*A:* 96 hours after application; cortical, endodermal, phloem, cambial, and ray cells flanking the phloem very highly meristematic, with beginnings of roots clearly delimited in outer ray derivatives. Ray cells flanking primary xylem and pith cells have not responded meristematically. *B:* 114 hours after application; roots clearly marked; inner ray cells and pith show virtually no meristematic activity but have progressed toward maturity.

FIG. 13.—Longitudinal section 136 hours after application; narrow band of lanolin mixture applied about midway of distance now showing roots. As usual there is as much response above line of application as below it, with reference to formation of roots, but formation of vascular strands derived from phloic and endodermal cells extends appreciably farther down stem than above it. In no case have tissue changes or tumor formation extended either as far up or down the stem as when lanolin mixture is applied to terminal cut surface, unless grown in saturated atmosphere or in darkness.

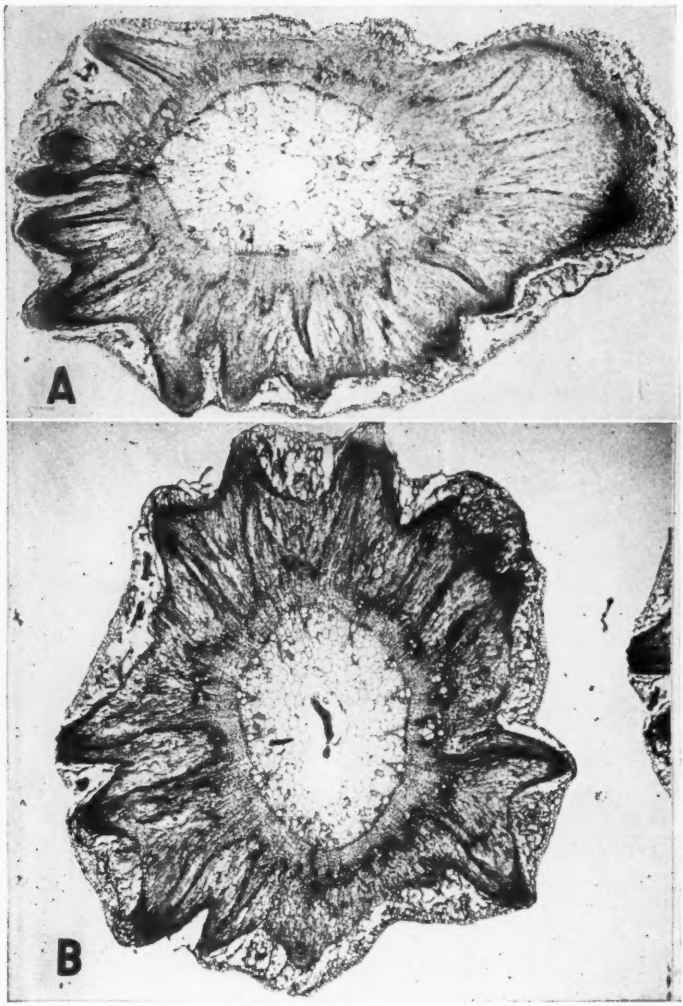

FIG. 14.—*A:* 9 days and *B:* 23 days after application, section through line of application. Pith at center has matured and begun disintegration in contrast to behavior of pith in root zone when mixture is applied to apical cut surface (fig. 5). Comparatively little meristematic activity.

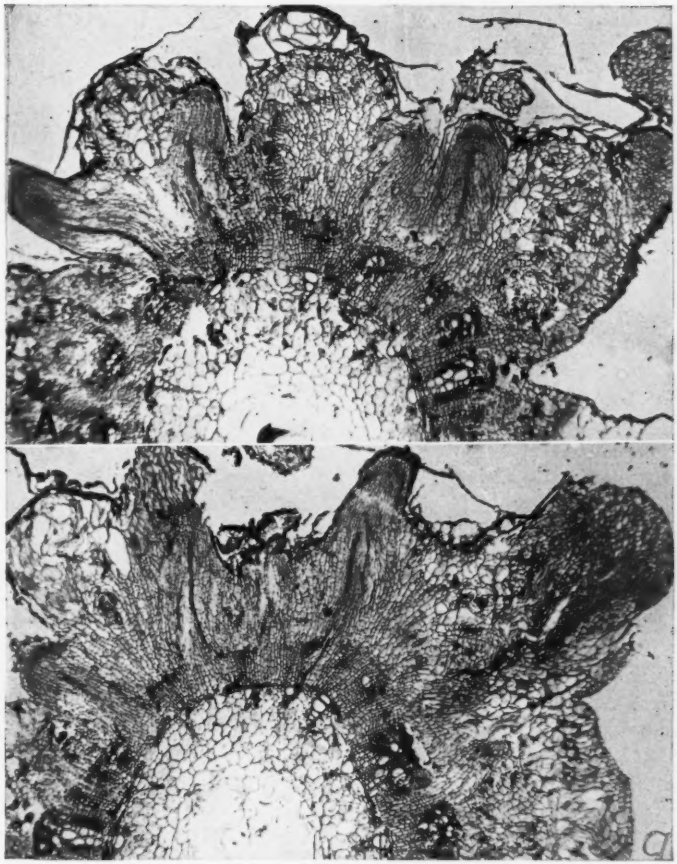

FIG. 15.—Section 66 days after lateral application. Parenchymatous tissues of vascular strands between adventitious roots and those of proliferated phloem are meristematic. These may continue proliferation over a long period of time, finally producing large tubercles and flanges of vascular and parenchymatous tissue which overgrow the adventitious roots (fig. 16). Pith not meristematic.

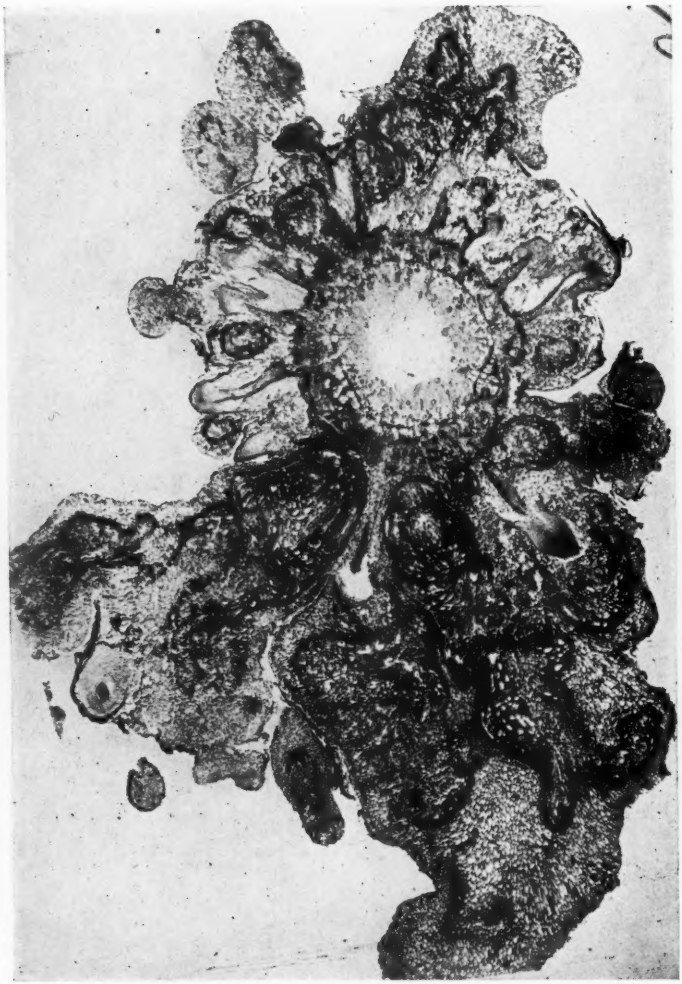

FIG. 16.—Transection through tumor showing several stages in development of masses of tissue from derivatives of phloem and derived endodermal tissues, in places completely enveloping the early formed adventitious roots. Other roots may be formed indiscriminately among these proliferating tissues.

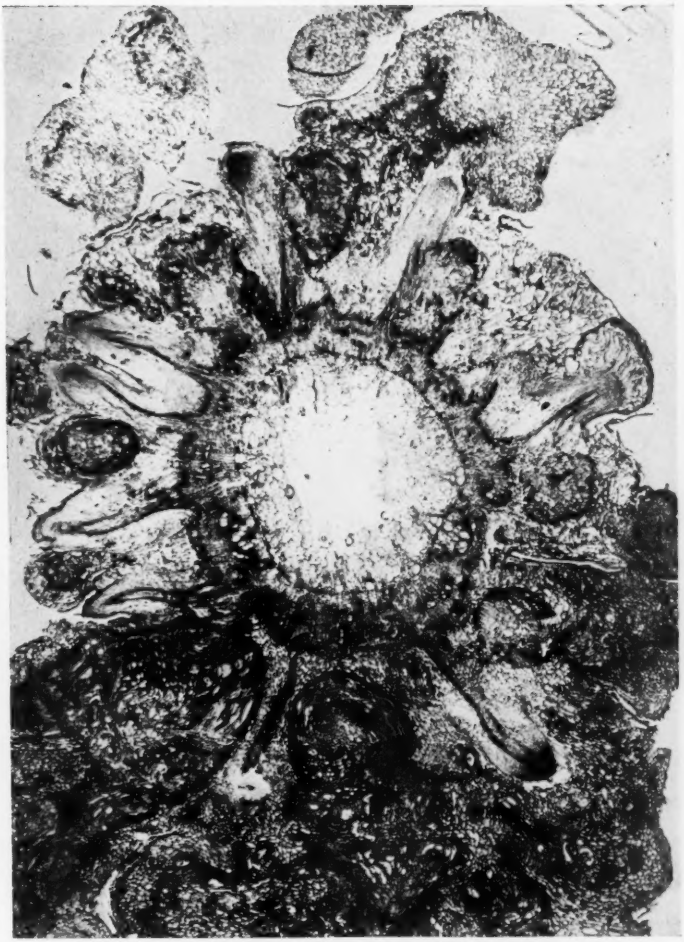

FIG. 17.—Same as fig. 16, enlarged. Cells of rays flanking primary xylem and of pith are non-meristematic and no derivatives from them have contributed to the large irregular masses of tumor.

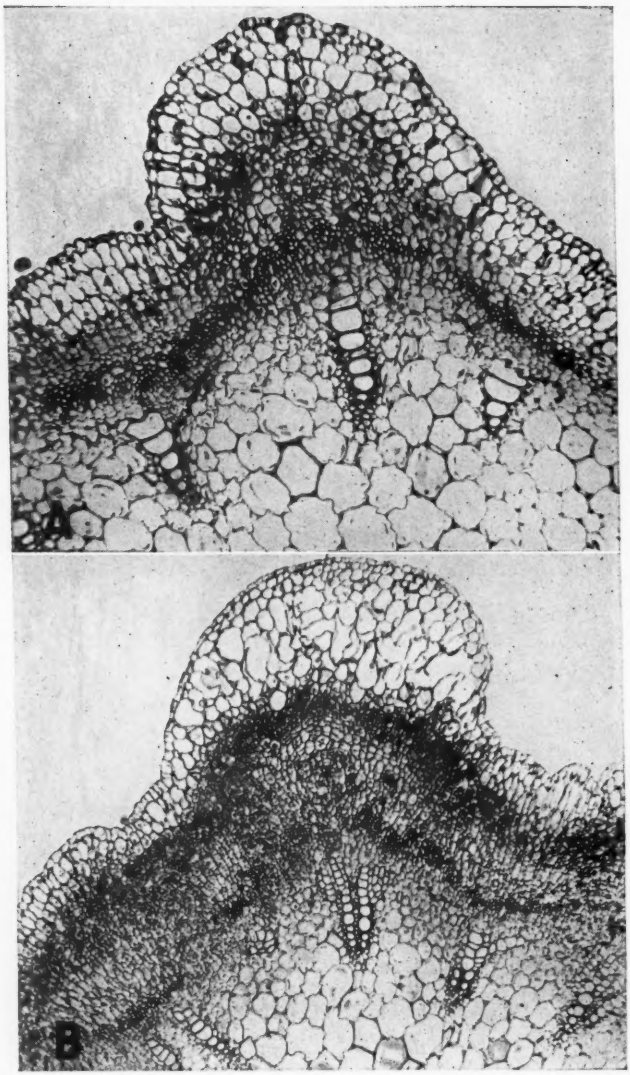

FIG. 18.—Transection of decapitated stem slightly below surface to which 1.5% indolebutyric acid in lanolin has been applied. *A:* 48 hours after treatment; inner cortical, endodermal, phloem, and ray cells actively meristematic. *B:* 72 hours after treatment; marked activity of endodermis. Vascular strands in proliferated phloem are being differentiated, as well as root primordia in rays, and, at places, from the endodermal derivatives. Cells of pith slightly meristematic.

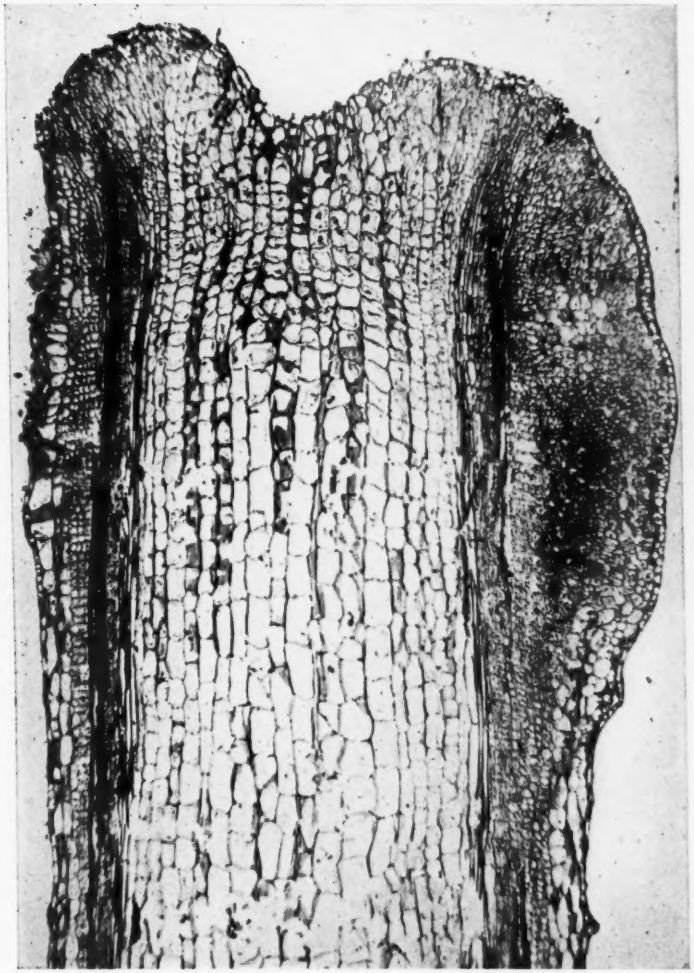

FIG. 19.—Longitudinal median section of developing tumor 96 hours after application of indolebutyric mixture. Cells of endodermis have proliferated markedly many cell layers below treated surface. Near top the cortical cells are active out to the epidermis. Those of pith are also active, but for far less distance down the stem.

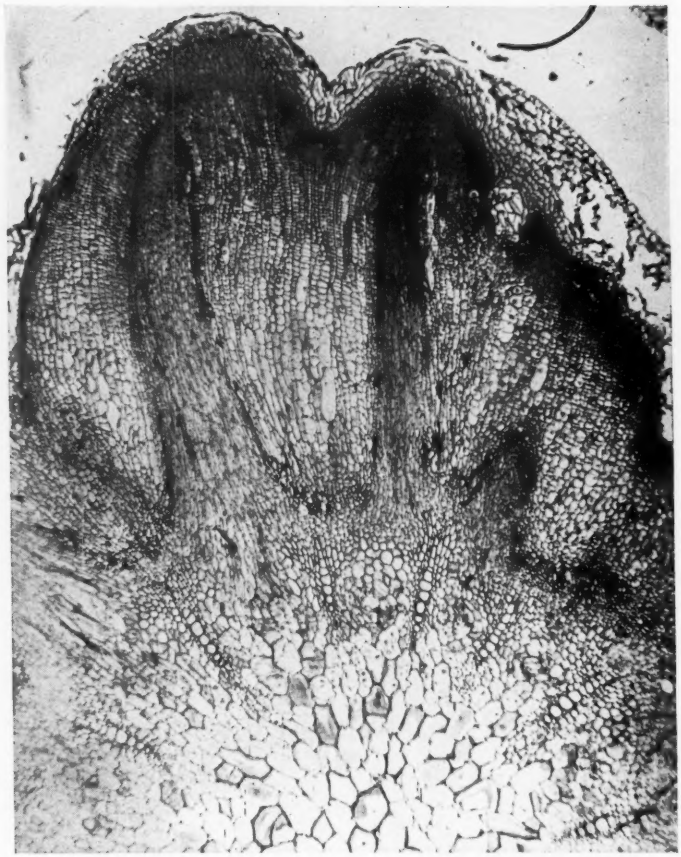

FIG. 20.—Transverse section through zone of adventitious roots 112 hours after treatment of cut surface with indolebutyric mixture. Cells of ray and some cells of pith are active, but less so than is usually true in stems treated with a 3% indoleacetic lanolin mixture.

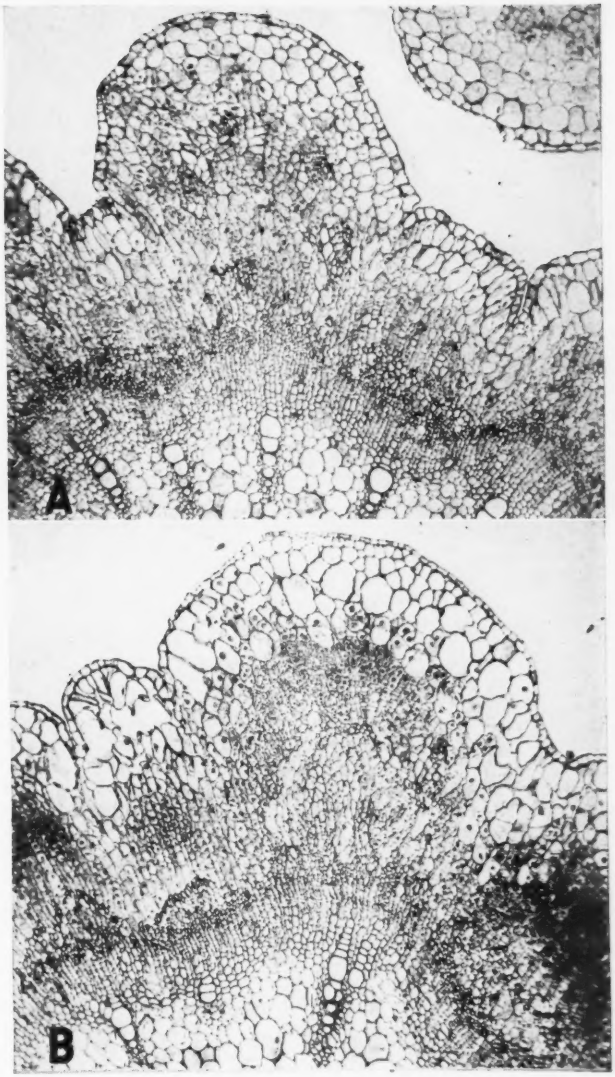

FIG. 21.—Transverse section of developing tumor 96 hours after treatment of cut surface with indolebutyric mixture. *A:* developing vascular strands from endodermal derivatives. *B:* another area in same section; root primordia being differentiated from endodermal derivatives over a phloem area.

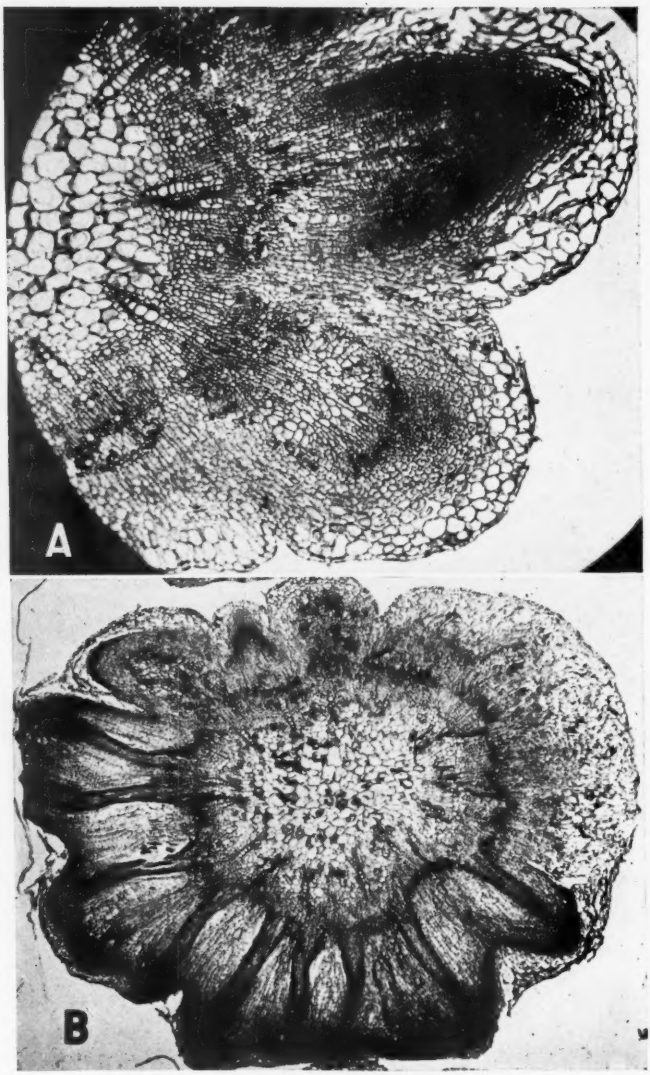

FIG. 22.—Transverse sections of tumor 162 hours after application of indole-butyric mixture. *A:* two bundles showing developments as in fig. 21*A*, *B*. Over one group a root initial has developed largely from endodermal derivatives; over the other a vascular strand is being differentiated partly from phloem derivatives, partly from endodermal derivatives, the latter still highly meristematic. In some cases root primordia arise from these exterior patches of endodermal cells. *B:* section through upper zone of adventitious roots differentiated mainly from ray cells, but some of them lie exterior to the phloem and are mainly endodermal in origin.

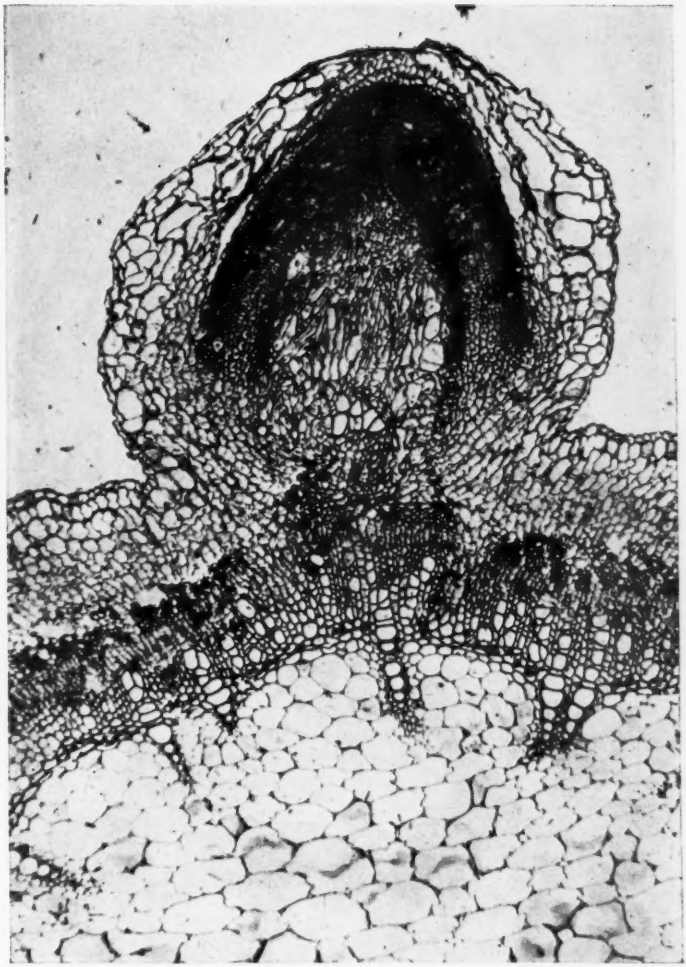

FIG. 23.—Transection of stem about 7 mm. below surface treated with indolebutyric mixture, and below main zone of adventitious roots nearer surface. Roots developed at lower levels generally occupy positions over the phloem instead of in the rays. Same age as fig. 22.

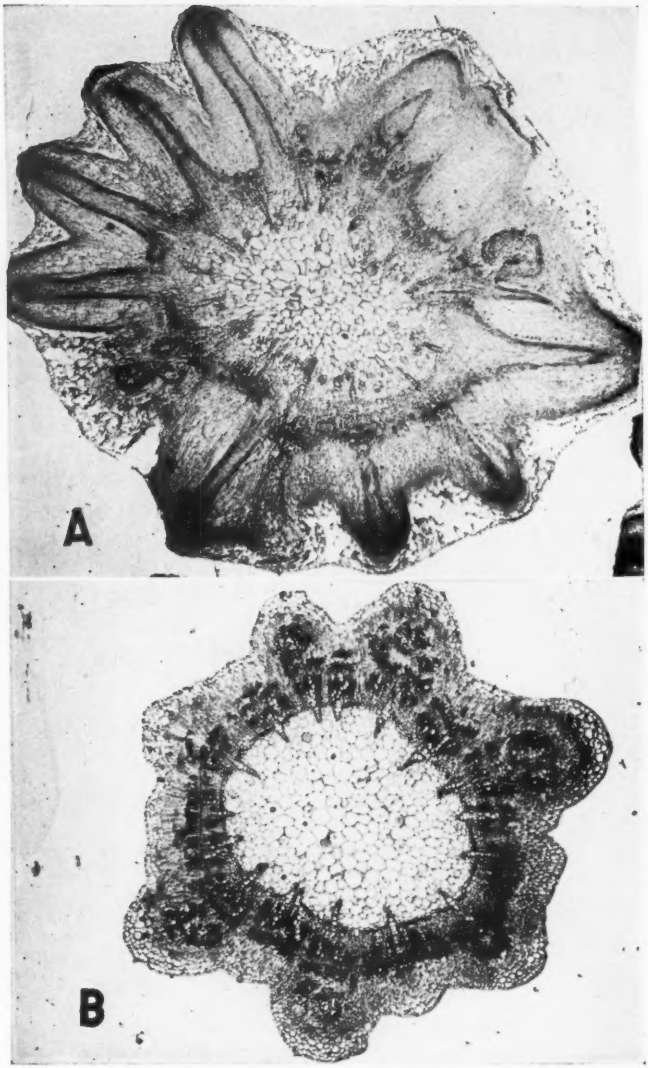

FIG. 24.—Section 216 hours after treatment of cut surface with indolebutyric acid lanolin mixture. *A:* through root zone near treated surface. *B:* about 2 cm. below. Outer vascular strands derived principally from endodermis over principal vascular bundles of stem. Other portions of stem have developed much as in an untreated stem.

FIG. 25.—Longitudinal section of apical tumor 216 hours after treatment with 1.5% indolebutyric acid in lanolin. Dark patches near top indicate level of treated surface. Above these the vascular apical tumor, derived mainly from pith derivatives, has begun to form. It develops almost exactly as do similar tumors resulting from application of 3% indoleacetic acid in lanolin (fig. 3). Below treated surface is an extensive root zone. The uppermost of these roots are generally initiated from endodermal and ray derivatives, sometimes two or more rays being involved in initiation of a single large root; lowermost ones generally initiated from cells over a proliferated phloem group of main vascular bundles of stem. Such a root is at the lower right.

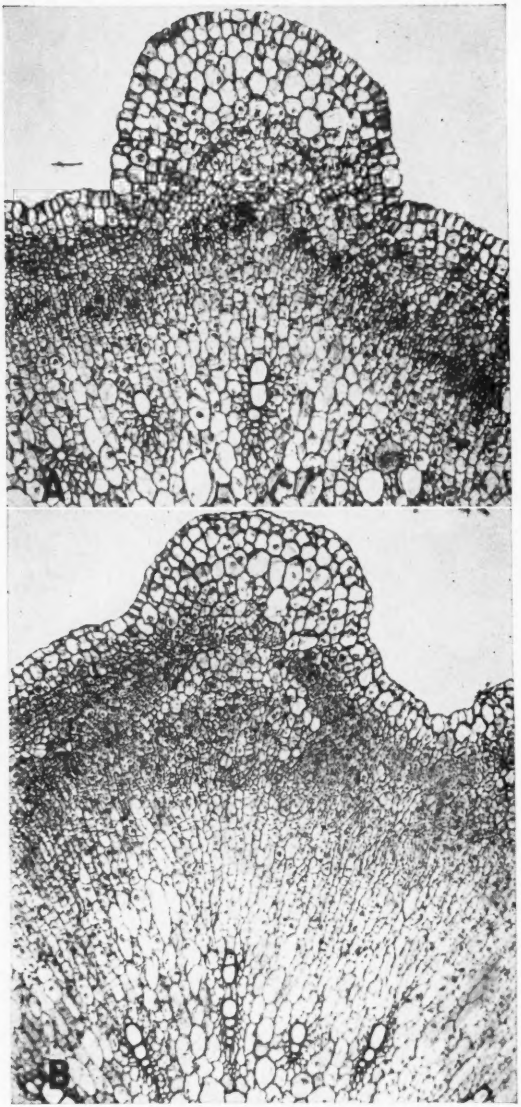

FIG. 26.—Transections of stems decapitated and treated with 0.5% naphthaleneacetic acid in lanolin. *A:* 48 hours after treatment; endodermal, phloic, cambial, and ray cells meristematic. *B:* 72 hours after treatment; outer cortical cells meristematic as well as those of endodermis. The meristematic activity of other regions except epidermis, pericycle, and pith is very great. Root initials evident in rays.

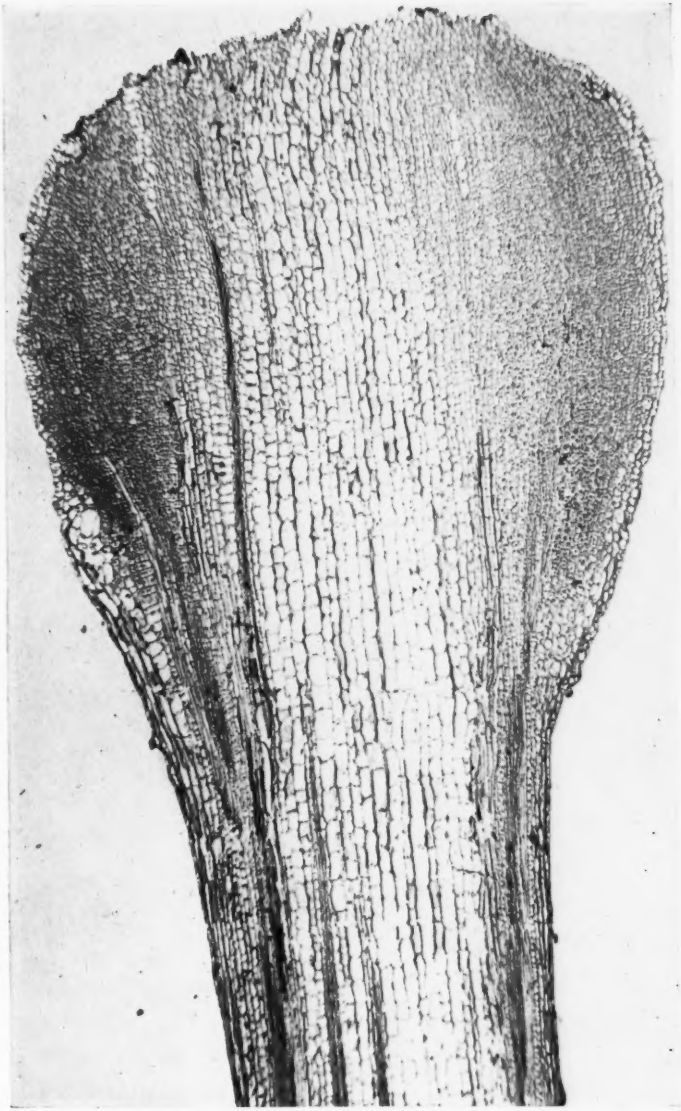

FIG. 27.—Longitudinal median section of apical tumor 72 hours after treatment of cut surface with 0.5% naphthaleneacetic acid. Activity of cortical, endodermal, phloem, ray, and cambial cells extends many cell layers down the stem. Pith shows very little meristematic activity except immediately below treated surface (fig. 29).

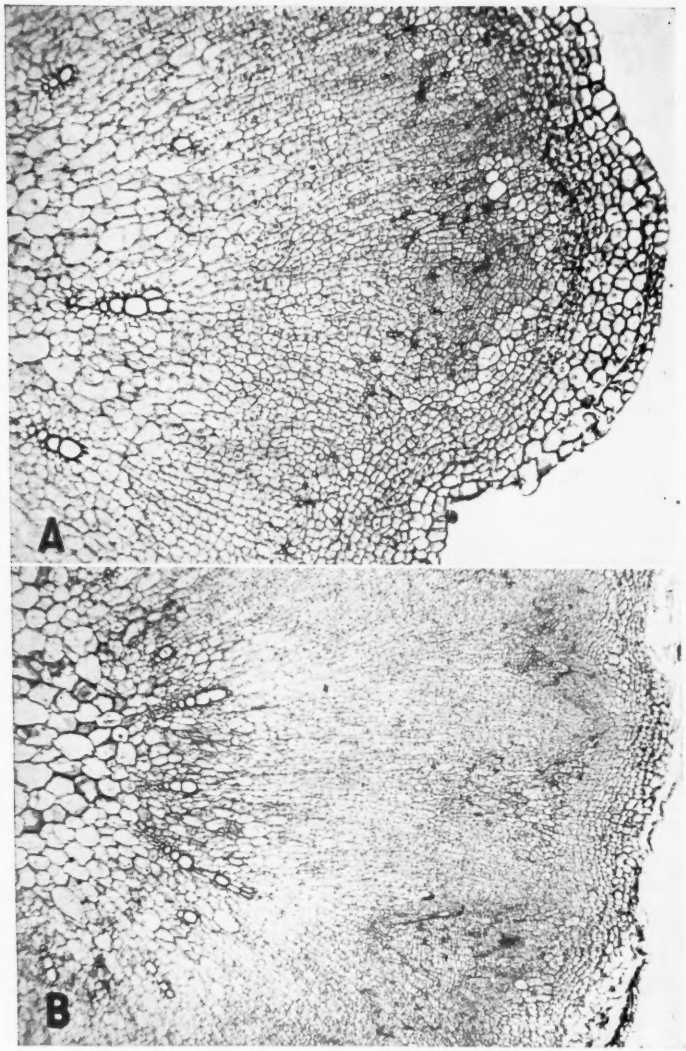

FIG. 28.—*A:* 96 hours after decapitation and treatment of surface with 0.5% naphthaleneacetic acid mixture. In addition to other tissues, some of pericyclic cells are meristematic. Pith shows almost no activity. Section about 150 μ below treated surface. *B:* similar section 120 hours after treatment. Root initials in rays clearly delimited.

FIG. 29.—Transverse section of decapitated stem treated with 0.5% naphthaleneacetic lanolin mixture, 80 μ below treated surface. Some of pith cells dead and disrupted, others highly meristematic and from these vascular masses are differentiating. Such activity is limited to layers near treated surface.

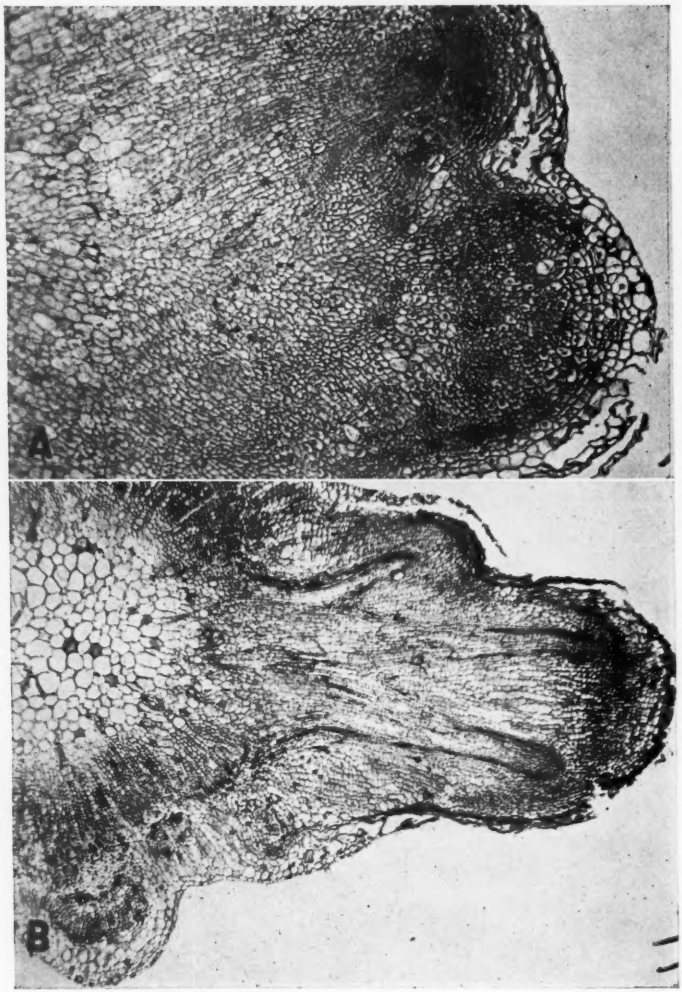

FIG. 30.—Two sections about 1 cm. below surface treated with 0.5% naphthaleneacetic lanolin mixture. *A:* 168 hours after treatment. A root is differentiating in a ray; over the phloem of an adjacent bundle the endodermis and other cortical cells are highly meristematic. *B:* similar situation after 32 days of development. A root complex has involved at least three adjacent vascular bundles. Structures such as these frequently occur in a circle around the stem, over some of vascular bundles, about 1 cm. below treated surface (fig. 32*A*).

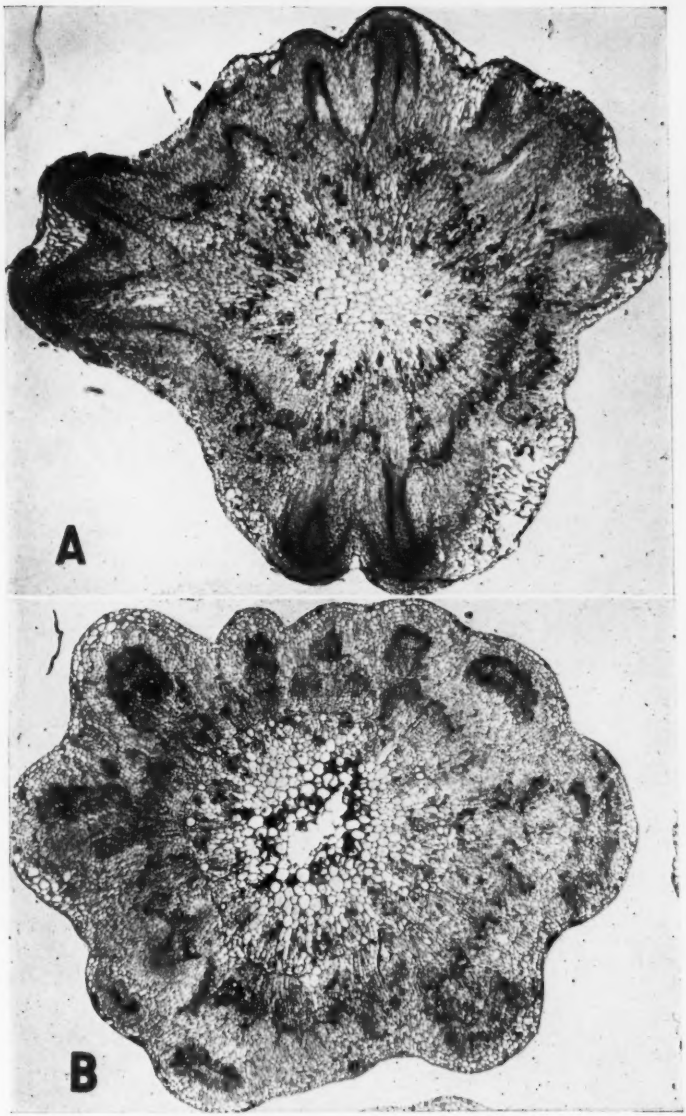

FIG. 31.—Section 32 days after decapitation and treatment of cut surface with 0.5 % naphthaleneacetic acid lanolin mixture. *A:* through root zone about 0.5 cm. below treated surface. *B:* just below root zone about 1 cm. below treated surface. Pith is meristematic immediately adjacent to primary xylem vessels. Over principal vascular bundles vascular strands in multiple series up to three or more, separated by parenchymatous cells, have differentiated mainly from endodermal and other cortical derivatives. Cortical cells generally more active than those at same level from treated surface of tumors resulting from 3% indoleacetic acid lanolin mixture or 1.5% indolebutyric acid lanolin mixture.

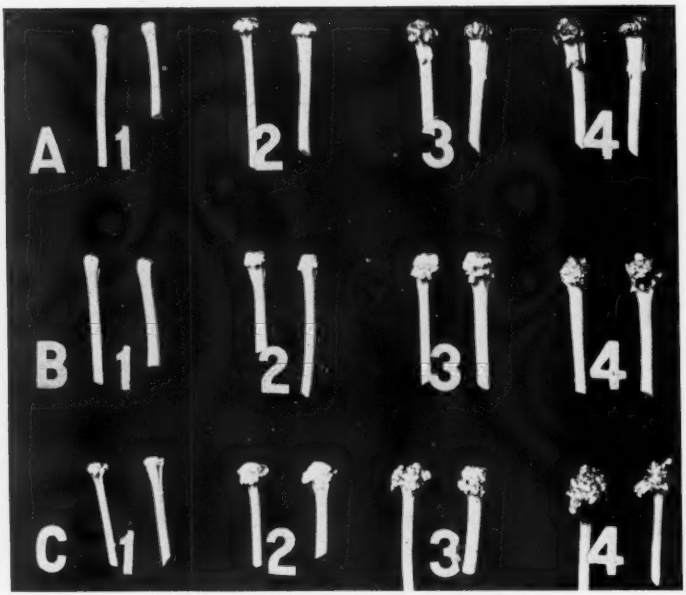

FIG. 32.—*A:* apical tumors resulting from application of 1.5% naphthaleneacetic acid; *B:* 1.5% indolebutyric acid; *C:* 1.5% indoleacetic acid, all in lanolin mixture. 1, 85 hours; 2, 7 days; 3, 14 days; 4, 21 days after application. Thickening of stem for about 1 cm. below surface of application is characteristic for tumors resulting from naphthaleneacetic acid in our cultures. Such tumors have never produced large apical tumors from proliferated pith and soon cease apical expansion. Development of large, vascular, apical tumors from pith characterizes effects resulting from indoleacetic and indolebutyric acid. Those here are just beginning to develop and during subsequent weeks would have attained several times their present size and height.

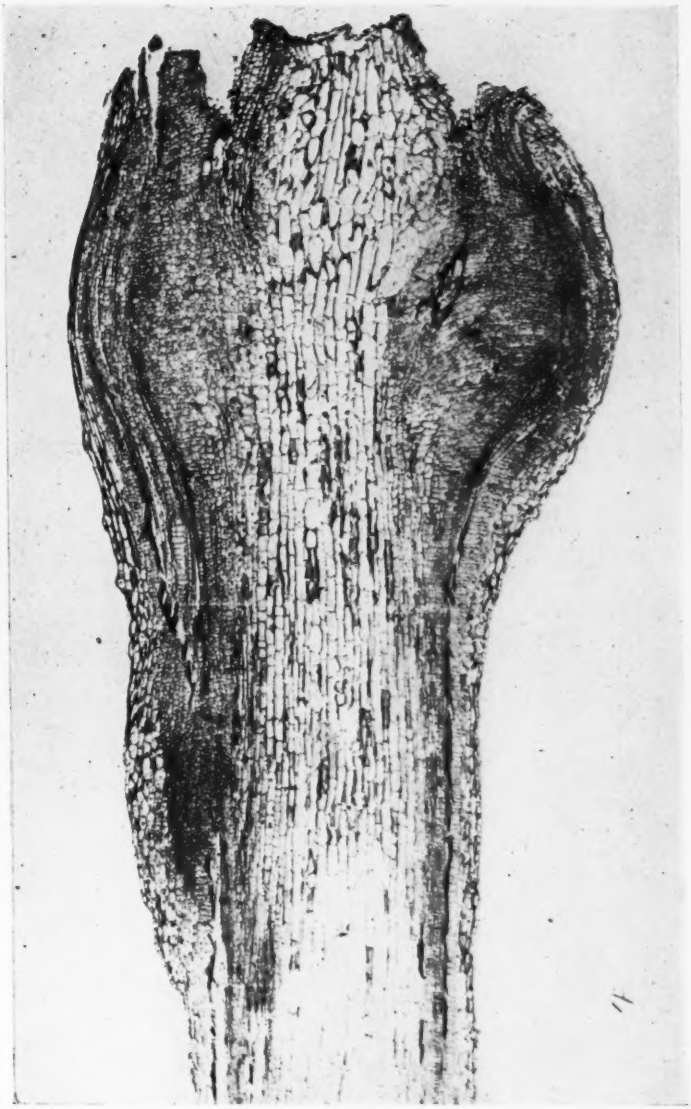

FIG. 33.—Four-day-old apical tumor resulting from application of 1.5% naphthaleneacetic acid in lanolin. Tissues near surface have been killed by this concentration, but several cell layers distant the responses are characteristic for this substance used in lower concentration (fig. 27).

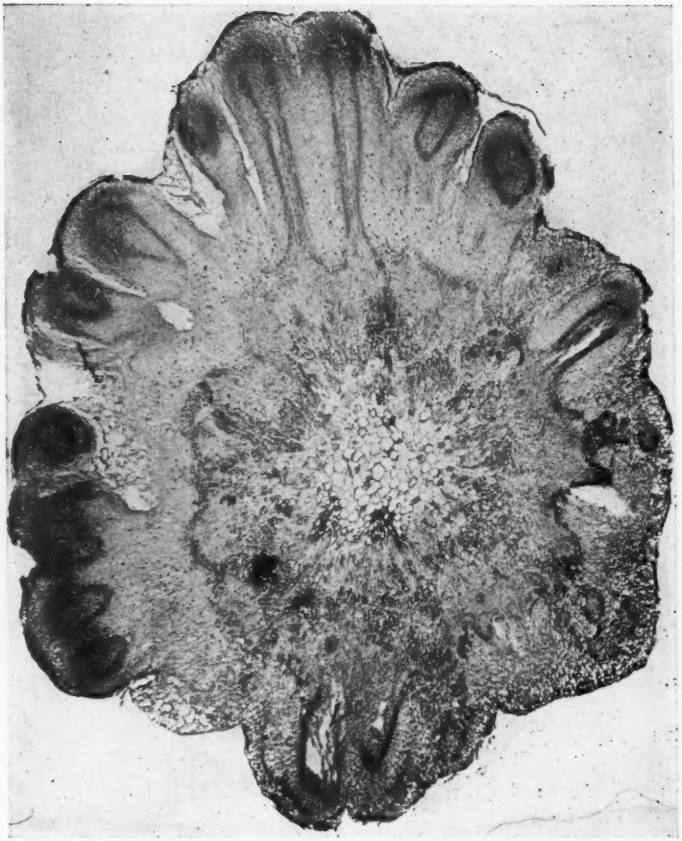

FIG. 34.—Section through root zone of apical tumor resulting from application of 1.5 % naphthaleneacetic acid lanolin mixture 21 days after application. It is more closely similar to tumors produced from 0.5% mixture of same substance than to tumors resulting from other two substances employed.

FIG. 35.—Tumors on bean pods 33 days after excising tip and treating with 3% indoleacetic acid lanolin mixture. In pod at upper left three seeds are maturing; at upper center, one seed; apical tumors smaller than those on pods in which seeds have aborted (lower row). Pod at upper right untreated. Tissues proliferate markedly only close to surface of application. Natural size.

FIG. 36.—Tumors on bean pods 33 days after slightly abrading epidermis at adaxial suture and applying along it a thin layer of 3% indoleacetic acid in lanolin. All seeds have ceased to develop. Proliferation has occurred close to surface of application only. Reduced about one-third.

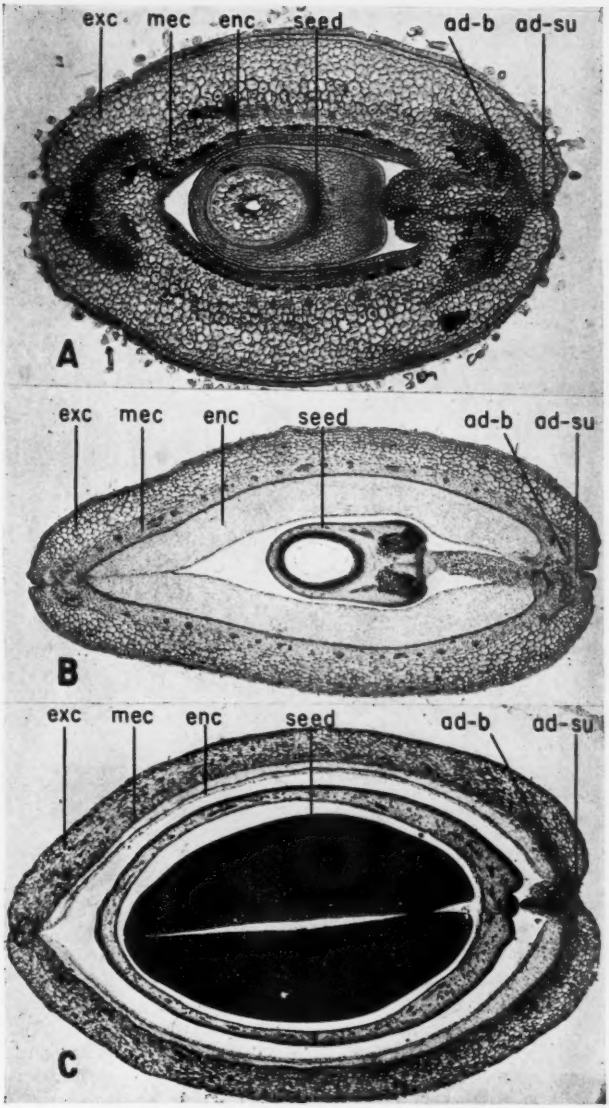

FIG. 37.—Transverse section of Red Kidney bean pods, untreated, through developing seed. *A:* shortly after fertilization; petals withered, entire pod about 1 cm. long, 1.5 mm. suture to suture diameter. *B:* several days later; pod about 5 cm. long, 5 mm. suture to suture diameter. Nearly all treatments of pods were made at this stage. *C:* nearly mature pod 12–14 cm. long, 1.5 cm. suture to suture diameter (*exc*, exocarp, non-vascular and parenchymatous except for layer of fibrous cells below epidermis; *mec*, mesocarp including vascular bundles, those at adaxial and abaxial sutures limited outwardly by fibers; *enc*, endocarp, parenchymatous and non-vascular, derived wholly or mainly as proliferation of inner epidermis; *seed*, seed; *ad b*, adaxial bundles, capped with fibers; *ad su*, adaxial suture). Various degrees of enlargement.

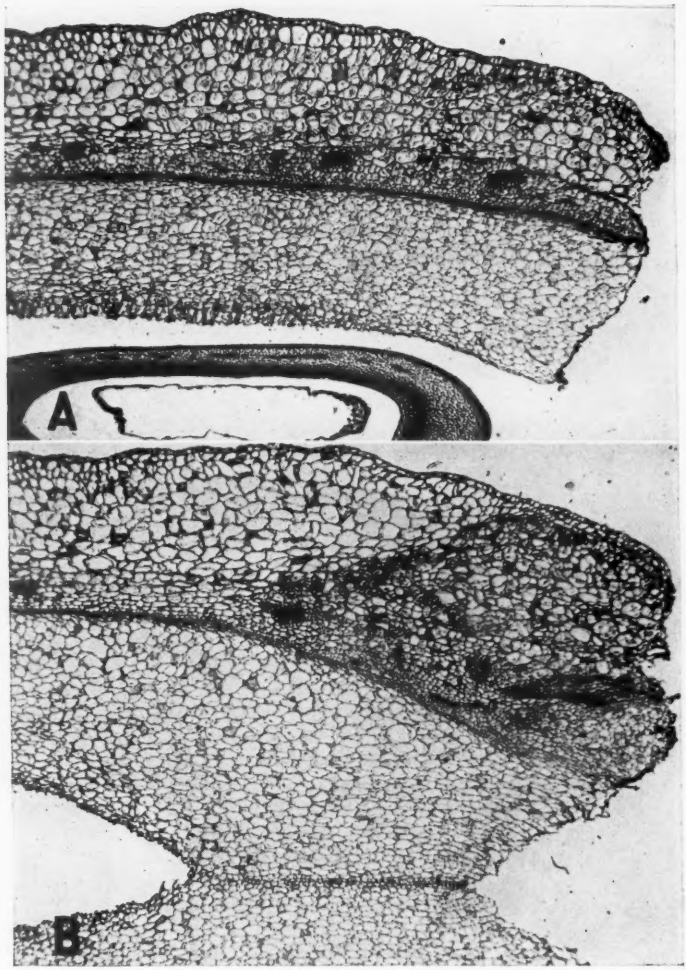

FIG. 38.—Pods 96 hours after having been cut off and treated with 3% indoleacetic acid in lanolin at cut surface. Sections cut longitudinally. *A:* pod with slight proliferation, limited mainly to parenchymatous cells of mesocarp. *B:* more usual condition, with marked proliferation of exocarp, mesocarp, and endocarp near surface of application.

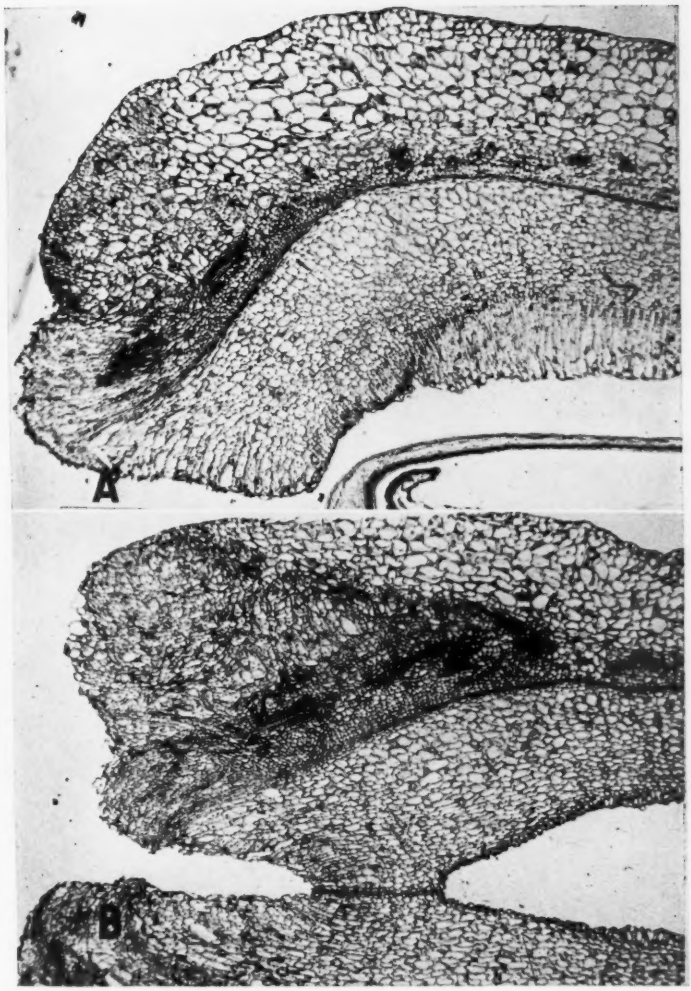

FIG. 39.—Similarly treated pods. *A:* 5 days after treatment. Some of proliferated cells of exocarp differentiating as vascular elements as are those of mesocarp, eliminating a clear line of demarcation between them. Endocarpic cells more highly meristematic near surface, but less active than other regions. *B:* 6 days after treatment, showing further progress in meristematic activity and differentiation of tissues. Derivatives of exocarp and mesocarp closing over endocarp.

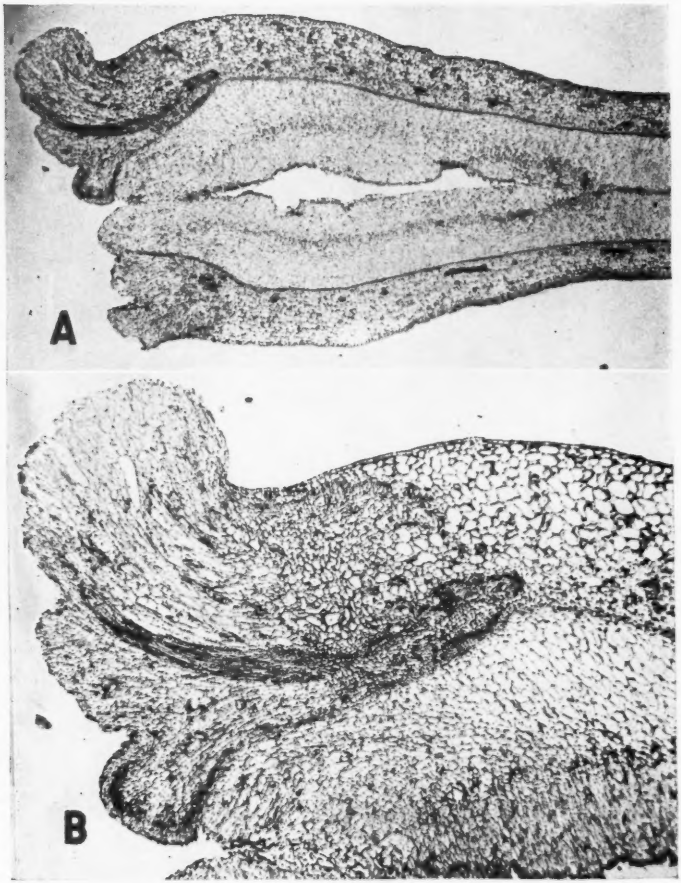

FIG. 40.—Similar pod 9 days after treatment. *B:* same as *A*, enlarged. Response of tissues obvious. Greatest and most active response is by exocarpic cells and that near surface of application. Marked response here of endocarpic cells at considerable distance from treated surface is probably result of accumulation of exudate in cavity of pod following amputation of tip and diffusion of indoleacetic acid through this over a large surface. No trace of vascular structures in endocarp.

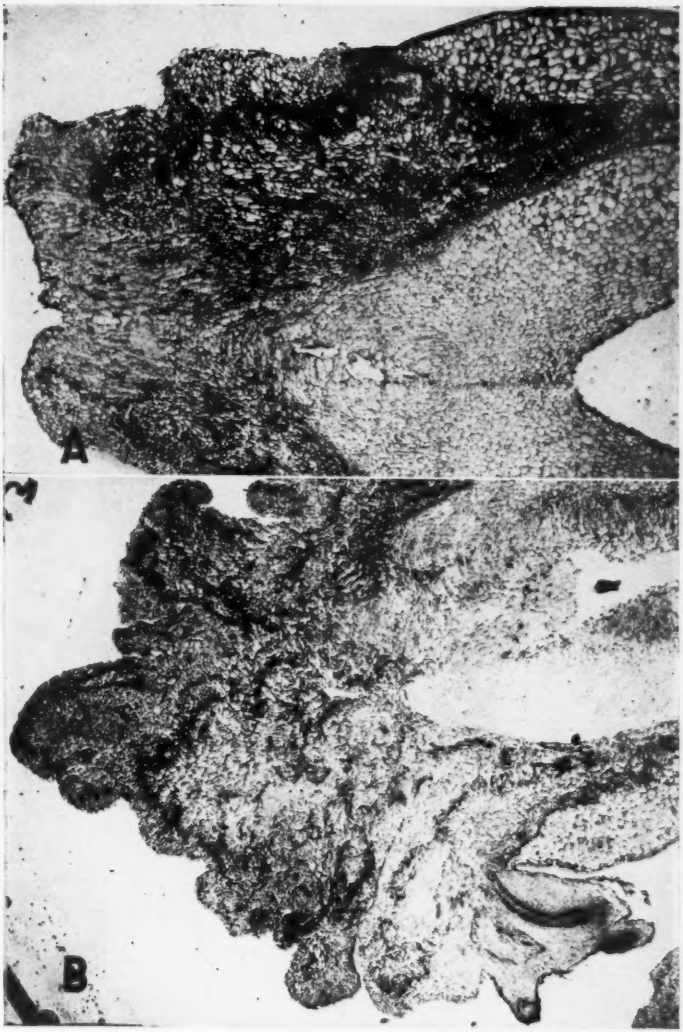

FIG. 41.—Similarly treated pods. *A:* 12 days, *B:* 20 days after treatment. Vascular strands, parenchymatous masses, and roots are differentiated from exocarpic and mesocarpic derivatives. Proliferation continues most markedly near surface and occurs irregularly, particularly under small films or masses of lanolin mixture which are carried out by proliferating and developing cells beneath them, thus making outer portions of tumor rough and tuberculated instead of smooth.

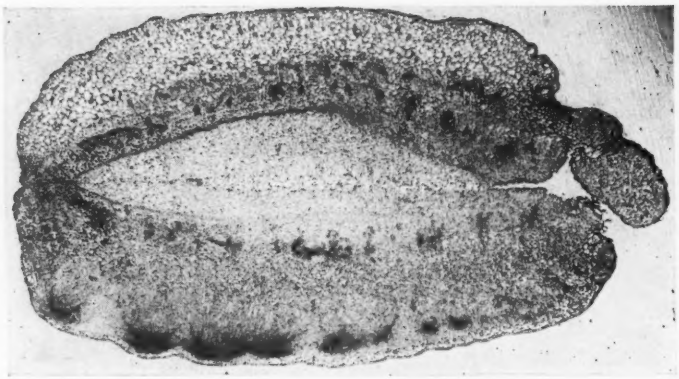

FIG. 42.—Pod with tip cut off, then treated apically. Transverse section 7 days after and practically at level of treatment, but just above adaxial bundles which would be at the right. Primordia of a number of roots differentiating from exocarpic derivatives.

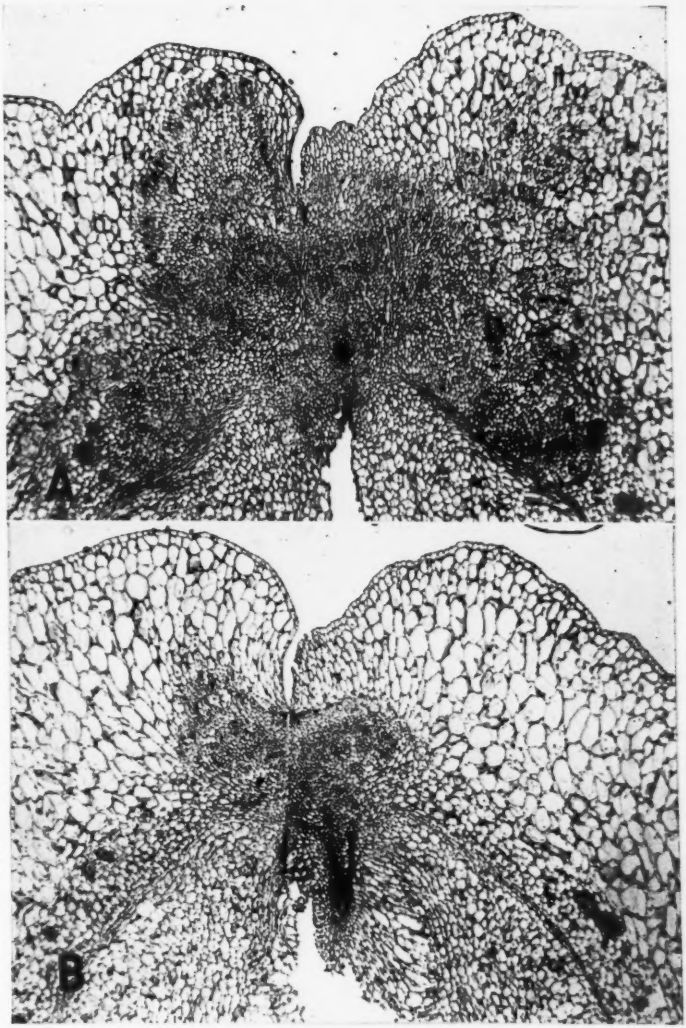

FIG. 43.—Same pod as fig. 42. *A:* about 300 μ below level of fig. 42. Exocarpic cells proliferating and differentiating; those of mesocarp show almost no response. *B:* 150 μ below *A*. Slight activity in exocarp only. In marked contrast to apically treated stems, cells of vascular bundles and those in their immediate vicinity show very little meristematic activity at other than very short distances from treated surface. Those derivatives away from lanolin mixture mature into vascular elements, although some remain as meristematic masses scattered between them. Those near lanolin mixture continue actively meristematic (figs. 50–53).

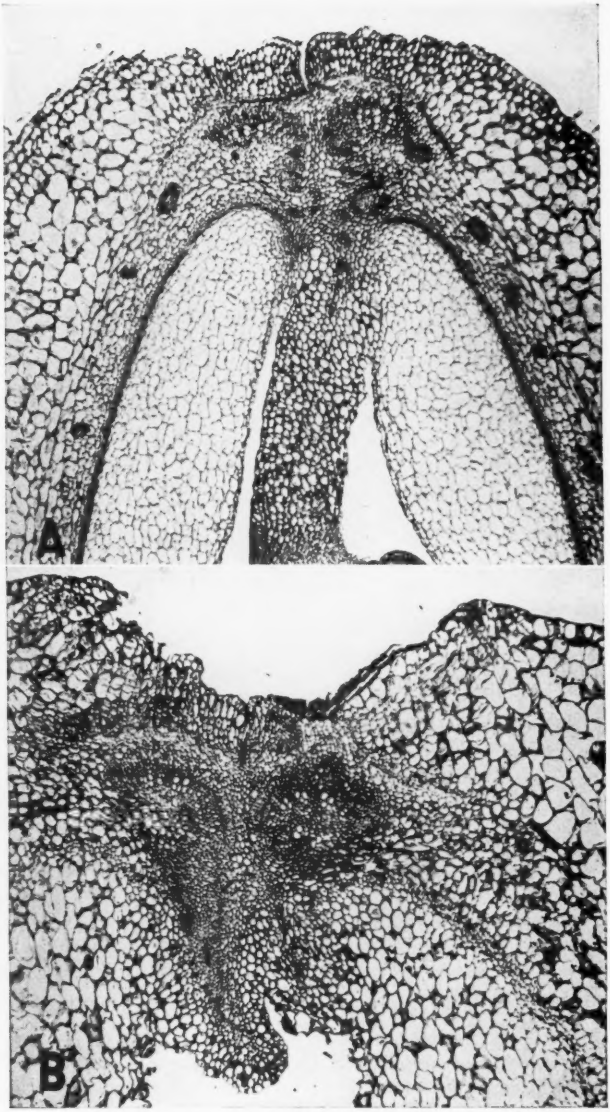

FIG. 44.—Pods treated along adaxial suture. *A:* check, abraded with emery cloth and fixed at once. *B:* 5 days after treatment with 3 % indoleacetic acid lanolin mixture. Cells of exocarp markedly meristematic; other tissues show no response.

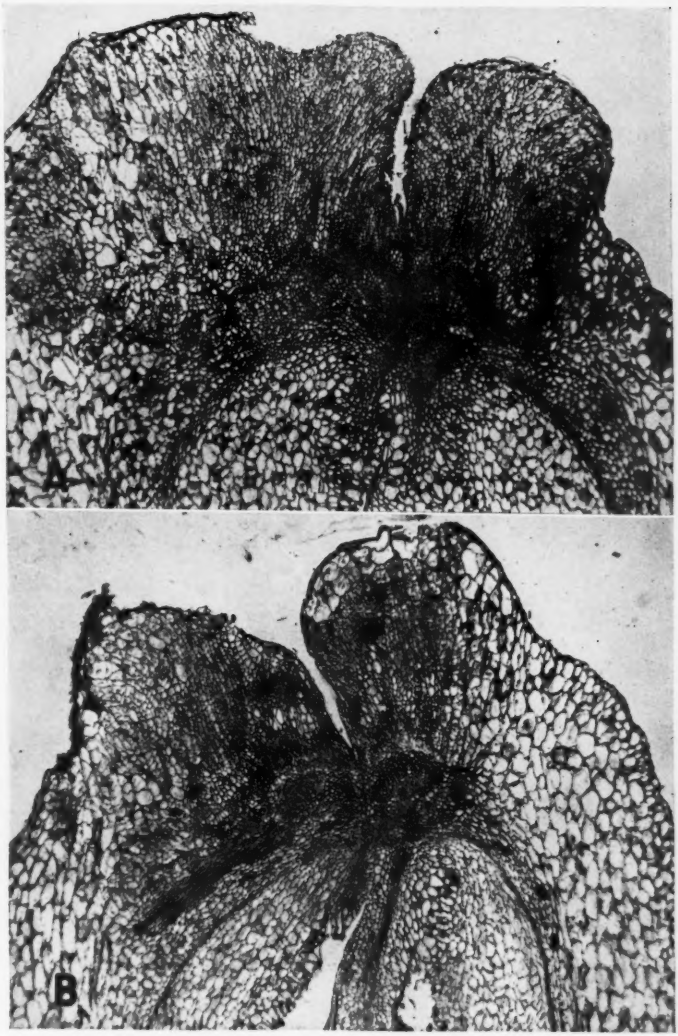

FIG. 45.—Two similar pods 6 days after treatment. Meristematic activity of exocarpic cells very great. Some derivatives are differentiating as vascular strands; others remain parenchymatous. Activity is greatest near surface but is still abundant to fibers of adaxial bundles. Mesocarpic and endocarpic cells not meristematic.

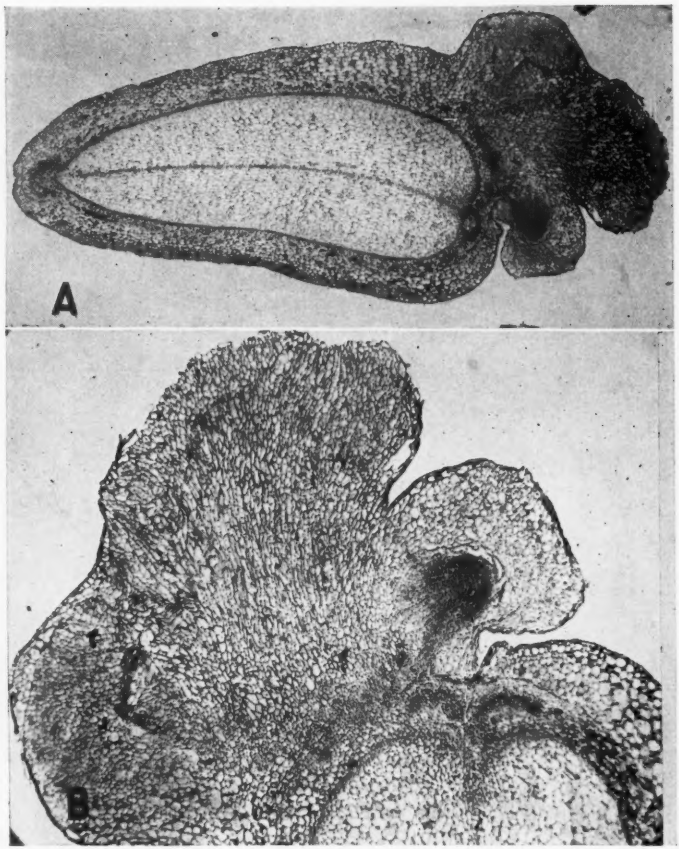

FIG. 46.—Similar pod 11 days after treatment. Proliferation almost completely limited to exocarpic tissues. In addition to vascular masses, a root has differentiated from derivatives. *B:* enlarged portion of *A*.

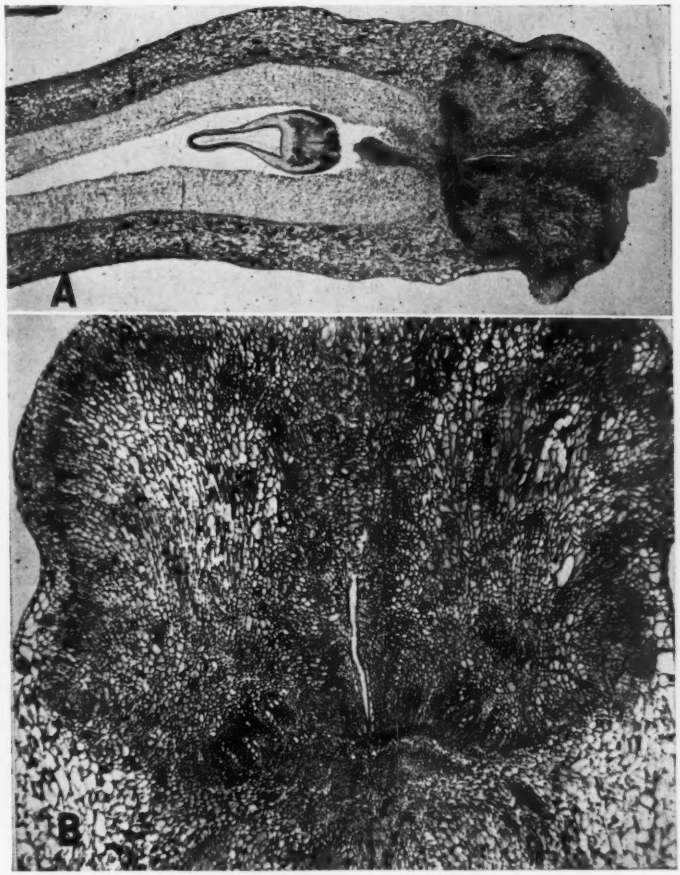

FIG. 47.—Similar pods 12 days after treatment. Proliferation almost exclusively exocarpic in origin, although there is some activity of parenchymatous cells of one of adaxial bundles just below and to left of large elongated cavity at lower center. Seed has aborted. *B:* enlarged portion of *A*.

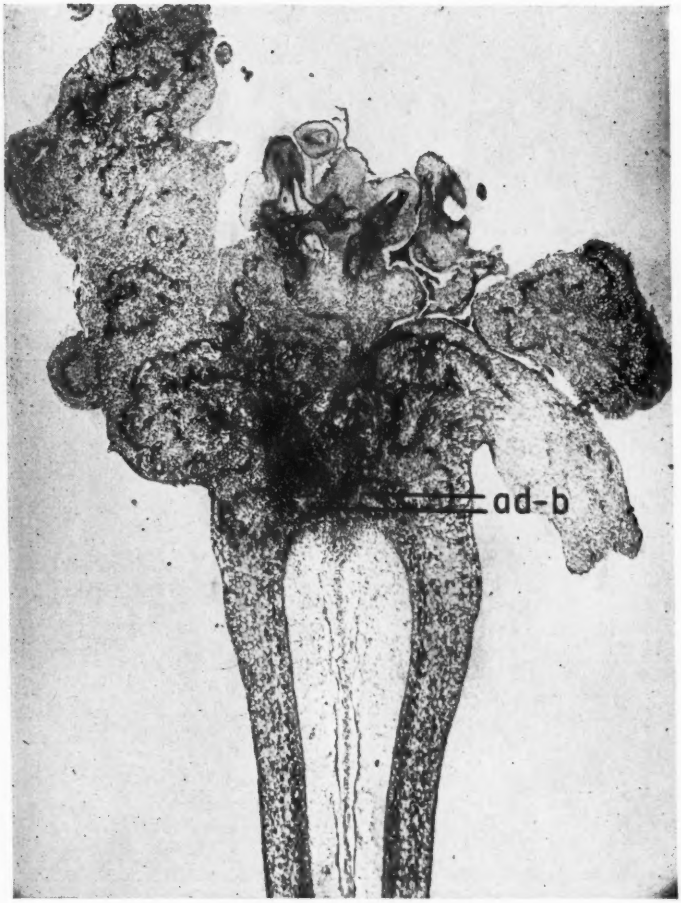

FIG. 48.—Similar pod 20 days after treatment. Practically entire tuberculated proliferation is from cells of exocarp. At upper center are several roots differentiated from same tissue. Adaxial bundles (*ad b*) show no meristematic response, nor does endocarp.

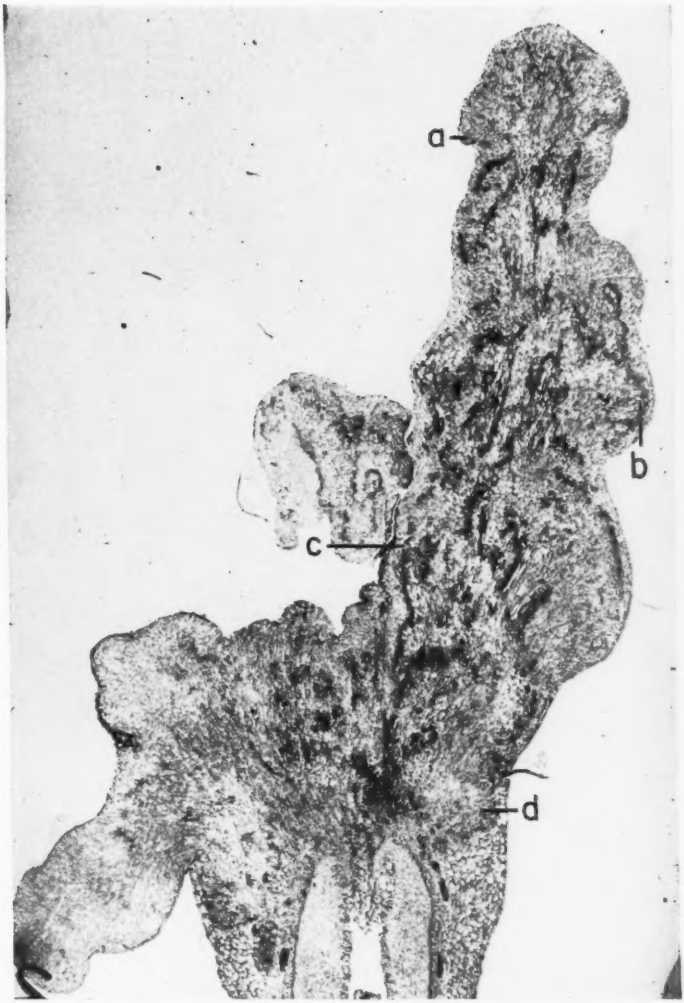

FIG. 49.—Similar pod 41 days after treatment. Most of tumor has been derived from proliferation of cells of exocarp and their subsequent differentiation, although a very small portion of it is derived from mesocarp near adaxial bundles. When collected, tumor was still actively developing, mainly peripherally, but many deep lying masses of parenchymatous tissue were highly meristematic. Letters *a–d* refer to regions shown enlarged in subsequent figures.

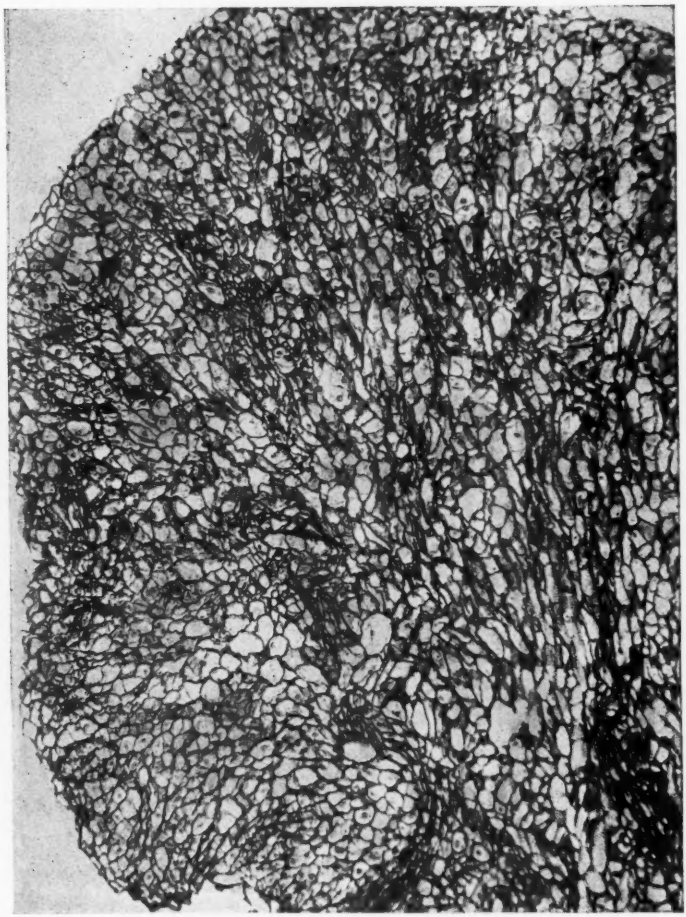

FIG. 50.—Enlarged portion of fig. 49 at *a*. Most cells of whole apical portion of tumor are actively meristematic. There are patches of parenchymatous cells which are more highly meristematic, while other groups are becoming differentiated as various types of vascular elements, sometimes as vascular strands consisting of distinct xylem and phloem portions with cambial zone between.

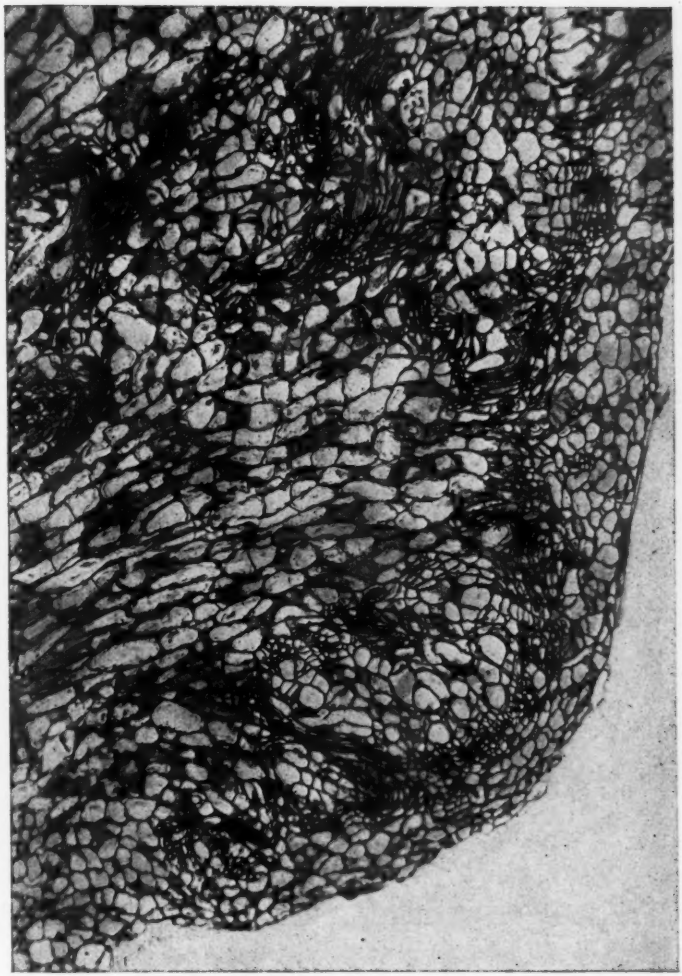

FIG. 51.—Enlarged portion of fig. 49 at *b*. A few cell layers below the surface large vascular masses have differentiated, with cambium-like zones between xylem and phloem portions. Still nearer the surfaces are patches of meristematic cells from which similar vascular masses separated by parenchymatous ones are later differentiated.

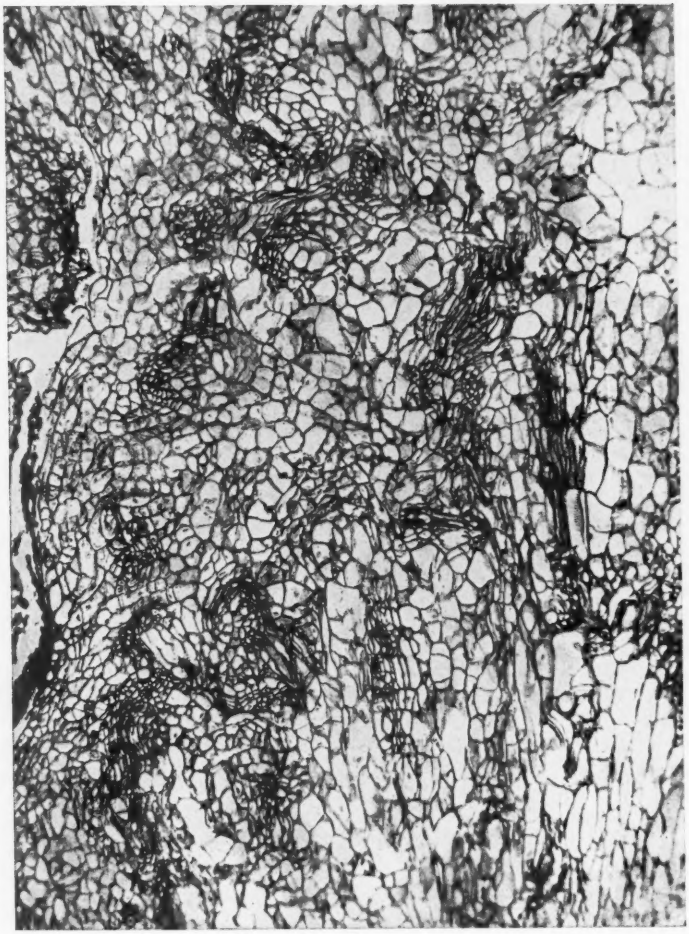

FIG. 52.—Enlarged portion of fig. 49 at *c*. This portion is similar to that at *b* except that vascular masses are smaller and more numerous, and some superficial cells which have died are being shed.

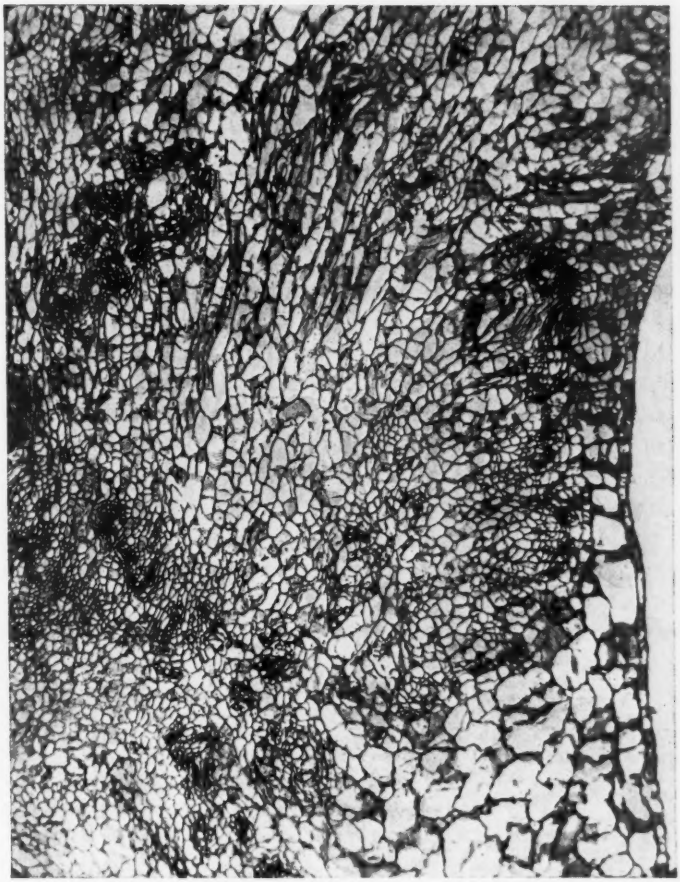

FIG. 53.—Enlarged portion of fig. 49 at *d*. In addition to same features as previously shown, at left lower corner the parenchymatous cells of one of adaxial bundles have proliferated, thus shoving some tracheids and vessels apart, and from some of the parenchymatous derivatives additional vascular strands and masses are being differentiated.

patches separated by patches of actively dividing cells. Such proliferation and maturation continued with great rapidity for many days, so that large roughened masses or tumors were formed. The more deeply lying cells of the mesocarp may not show any meristematic activity for several weeks after application of the mixture. In other instances after five to seven days the parenchymatous cells lying between the vascular bundles of the mesocarp or some of the parenchymatous cells of the adaxial bundles may become meristematic, and from such derived cells additional vascular patches, interspersed with highly active parenchymatous cells, are differentiated. Root primordia were frequently differentiated from the exocarpic derivatives (fig. 46). After several weeks of development, a section through a tumor (fig. 49) shows that most of its bulk has been derived from continued proliferation of the derivatives of the cells which composed the exocarp, although some of the parenchymatous cells of the mesocarp may also have proliferated and differentiated. Immediately under the surface there are bands and patches of highly meristematic cells and just underneath these are enlarged parenchymatous cells, patches, and strands of vascular elements in every possible degree of differentiation, as well as patches of actively dividing parenchyma. Thus activity continues indefinitely throughout the tumor, although this is most intense near the surface and is especially marked just beneath small bits of the mixture which remain on the surface of the tumor and are carried outward by the differentiation of tissues beneath them.

Discussion

The results thus far obtained indicate that any parenchymatous tissues of the epicotyl may become meristematic when indoleacetic acid is applied to them, and of these the derivatives may differentiate into vascular structures, with the exception of the epidermis (including under this term the endocarp of the pod as here delimited) and the possible exception of the cells of the pericycle. Considerable further study with reference to the latter is necessary.

It is obvious that the same general types of responses follow the application of indoleacetic, indolebutyric, or naphthaleneacetic acid, although there is an appreciable difference in the degree or intensity

of response of the several tissues in relation to these specific substances. There is more difference in response with respect to the kind of substance employed than to the concentration of a single substance when it is applied over a rather wide range of the higher concentrations in a mixture with lanolin. It is, of course, difficult to say exactly just what may be the concentration of the substance which reaches any cell or group of cells when applications are made in this manner. The substance no doubt is soluble and diffuses from the lanolin into and among the cells of the plant, but the rate at which this occurs is difficult to determine. It is very doubtful, however, whether the increased rate of root growth which takes place when the tumors are surrounded by a saturated atmosphere, or some other wet medium, is brought about by an increased concentration of growth substance acting immediately upon roots, for when stronger concentrations are applied directly either in a relatively dry or a saturated atmosphere the roots are frequently killed rather than stimulated to greater development.

The experiments dealing with the lateral application of the mixture are suggestive in relation to the course of travel of the indoleacetic acid through the plant. It will be recalled that when the stems are decapitated and the mixture applied to the cut surface there is marked stimulation and proliferation of the pith cells adjacent to the primary xylem vessels within 48 hours for some distance down the stem, and that this proliferation continues and increases as time elapses until all the pith cells become involved. Apparently some of the indoleacetic acid may travel in vessels of the xylem. In the case of the lateral applications, however, the pith cells rarely show any activity whatsoever at any time, and even the ray cells adjacent to the xylem proliferate but little or not at all, except when the plants are grown continuously in darkness. The outer ray cells lateral to the phloem do proliferate and produce roots very abundantly. The cambium and most of the parenchymatous tissues exterior to it proliferate much as they do when the mixture is applied terminally, but centripetal to this region there is practically no proliferation. Once proliferation has begun much of the continued division and differentiation of cells is peripheral. But it is by no means all peripheral, for even in the largest and oldest apical

tumors, and in the lateral ones also, patches of highly meristematic cells are found deeply imbedded among the vascular masses. There is little doubt, however, that a continued diffusion of the indoleacetic acid to the cells is essential, as is indicated by the more rapid building up of tubercles when there is a bit of the mixture at its tip, even though the amount may be very small. If this material is removed any particular tubercle may cease development completely, although in many instances new tubercles will start into active growth in the depression between the larger ones; and so far as can be detected there is no mixture at their tips. Diffusion through the meristematic cells must take place if proliferation of cells is used as a criterion for the presence of indoleacetic acid, because such patches of cells are relatively large and at considerable distance from any vascular element. Conduction through tracheids or other xylem or phloem elements which are differentiated throughout and among these meristematic islands may transport it more readily.

Why the rhythmic development occurs both in the apical tumors and particularly strikingly so in the lateral tumors is yet without clear explanation, as are many other occurrences, such as the very rapid early development of the roots, then their almost complete cessation of growth unless the atmosphere is very moist. If the atmosphere is nearly saturated, development is almost continuous from the time of application of the mixture, and the number produced is greatly augmented. Whether in differentiation the formation of many or few roots is to take place, or whether large numbers of vascular strands are to be differentiated from the endodermal and phloic derivatives, or whether there is to be a continued proliferation and the building up of large masses of vascular tissues as tuberculate masses may well depend upon specific environmental conditions which have escaped detection or delimitation because of the relatively generalized rather than the precisely controlled conditions under which the plants were grown. Certain it is that the generalized patterns are similar under a wider range of environment, but it is equally certain that the degree of expression of any particular response varies under different environments, such as variation in moisture, temperature, darkness, exposure to light, variation in

light quality and intensity, nutrients, and other factors. All these remain for exact testing.

The experiments on the pods with the 3 per cent indoleacetic lanolin mixture indicate that the general types of responses are similar to those of stems. As already suggested, the response of the endocarp closely parallels the response of the epidermis so far observed, whether on pod, stem, or leaf, except that its meristematic activity is greater. The responses of the mesocarp are similar to those of the vascular bundles and rays between them, and those of the exocarp resemble those of the cortical tissues. While specific experiments have not yet been precisely conducted to test the point, the failure of the seeds to continue to develop when applications along the adaxial suture are made may be related to a diversion of the food supply from the seeds to the developing tumors. If there is but slight tumor development, seeds often do develop; conversely, the generally smaller apical tumor formed when the pods are cut and treated apically and the seeds continue to develop may well be related to a smaller amount of foods or nutrient materials which reach the cells composing the tumor.

Before it is possible to discuss the direct qualitative effect of these growth promoting substances on cells and cellular differentiation, some clearer and more definite means for exact quantitative determination of these substances, particularly in the tissues, must be found. The present method of attempting to estimate them quantitatively on the basis of the intensity or degree of reaction of specific cells or tissues is often not reliable, and is unsatisfactory because such intensity or reaction is dependent on various environmental factors as well as on the quantity of growth substance present or supplied. The variation which exists among the several individuals of any lot of plants grown from the purest strains of seeds now available, or among individuals which have been propagated vegetatively, is relatively large. Further experimental evidence, gained under precisely controlled experimental conditions, is necessary before many of the present or even future interpretations and hypotheses can have much real meaning.

The close similarity of tumors resulting from the application of

indoleacetic acid lanolin mixture and those produced by an extract from *Bacterium tumefaciens* has been pointed out previously by BROWN and GARDNER (3), and some of the histological similarities of the two types of tumors have been recorded (5). The close resemblance of the tumors resulting from application of lanolin mixtures of indoleacetic acid, indolebutyric acid, or naphthaleneacetic acid shows that many of the responses made by the Red Kidney bean are not absolutely specific or diagnostic for any one of these substances. Such additional evidence as has been gained, however, seems to strengthen the suggestions of BROWN and GARDNER.

Summary

1. Apical tumors developed following the application of 3 per cent indoleacetic acid lanolin mixture to cut surfaces of decapitated bean plants may continue development for periods longer than six months and attain diameters of 2 cm. or more. Such tumors are developed largely from proliferations of the cells composing the pith, some of which differentiate into vascular elements variously arranged and anastomosed; others remain more or less actively meristematic.

2. Large, irregular vascular tumors are developed when similar mixture is applied as a narrow band encircling an uninjured young internode of the stem. Response is about equal above and below the band under average greenhouse conditions.

3. The stems to which such bands have been applied when grown under average greenhouse conditions are not retarded in their apical extension but grow and fruit as well as untreated stems under comparable environmental conditions. The pith of stems treated in this manner shows virtually no meristematic response, but develops as does that of stems which have been untreated. The roots developed are related to the rays in the same manner as those developed in apical tumors of decapitated plants.

4. There are more or less definite rhythmic periods of rapid development and comparative quiescence in the development of lateral tumors through an extended period. The irregular lateral outgrowths or tubercles of lateral tumors are developed mainly from derivatives of the phloem and to some extent from the cortex.

5. Apical tumors resulting from the application of 1.5 per cent indolebutyric acid in lanolin to decapitated stems were similar to those resulting from applications of 3 or 1.5 per cent indoleacetic acid mixture. They differ mainly in having somewhat fewer, thicker roots in the upper zone, more roots produced 1 cm. or so below the treated surface, and several tiers of roots developed between these two regions. There is slightly greater delay in the formation of the apical tumor above the treated surface, but eventually it develops to as large size, although its surface is generally smoother and less tuberculated than that developed following treatment with indoleacetic mixture. There is marked swelling of the stem and differentiation of vascular strands for several centimeters below the treated surface.

6. Apical tumors resulting from the application of 0.5 per cent naphthaleneacetic acid in lanolin differed from those following the application of the other two substances in having greater, more uniform diameter for a distance of about 1 cm. below the cut surface, where a circle of large root primordia is formed, each primordium over one of the main vascular bundles. Below these primordia the tumor tapers sharply to a much smaller diameter, giving it a distinct shouldered appearance. Below it prominent ridges extend for several centimeters down the stem. There is much less proliferation of the pith; only a slight tumor is developed above the surface of application.

7. Apical tumors resulting from the application of 1.5 per cent mixtures of each of the substances to individual stems showed greater differences than tumors produced following the application of weaker or stronger concentrations of the same substance.

8. Applications of 3 per cent indoleacetic mixture to the cut surfaces of partially mature pods which had their tips removed resulted in the production of very large vascular apical tumors and roots. The tissues composing such tumors were derived mainly from the proliferated exocarp and mesocarp of the pod. The endocarp which in untreated pods is principally derived from proliferated epidermal cells becomes somewhat more active in treated pods, but forms little or no part of the apical tumor; nor have vascular elements differentiated from this proliferated tissue been observed.

9. The large apical tumors are built up from derivatives of cells which originally were near to or but a relatively few cell layers away from the treated surface, a far shorter distance than is the case in stems.

10. In at least half the apically treated pods the seeds abort. If the seeds continue to develop, the apical tumor produced is generally smaller than if they abort.

11. When the epidermis at the adaxial suture of partially developed pods is slightly abraded and 3 per cent indoleacetic lanolin mixture applied, large vascular tumors are developed from the proliferated exocarp and to a far lesser degree from the mesocarp. In such pods most of the seeds abort.

12. The quantitative response made by stems or pods when treated with any one of the three substances used in mixture with lanolin was influenced more pronouncedly by small variations in the environment or the state of vigor or development of the plant than by comparatively great variations in the concentration of the particular mixture used or in the amount of the mixture applied. There was less difference in this respect in the qualitative than in the quantitative response.

13. Caution is required, therefore, in any endeavor to make a quantitative estimate of the amount of any of these substances which may be present in the living tissues of the plant if such estimate is based on a quantitative response of tissues in close proximity to the place of application or distant from it.

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MORPHOLOGY OF CUPRESSUS ARIZONICA: GAMETOPHYTES AND EMBRYOGENY

CLIFTON C. DOAK

(WITH FOURTEEN FIGURES)

Literature and object

BUCHHOLZ (1–5) has made wide use of comparative embryogeny to determine nearness of kinship among conifers. The extensiveness of his work was made possible by the perfection of a dissection technique (4) which permits the examination of embryos and coiled suspensor systems without the tedium of sectioning, and without the difficulty of subsequent interpretation from fragments which sectioning of coiled structures entails. He has made extensive use of the stages between the organization of the proembryo and the organization of the tissue regions of the embryo. Both in the works of BUCHHOLZ and in the recent summary of conifer embryogeny by SCHNARF (8), comparative embryogeny is found to be of taxonomic value. This emphasizes the necessity of our acquiring a wide knowledge of embryogeny before we can consider complete the work arranging the constituents of the Coniferales into a natural system.

Information regarding representatives from most of the genera is now available, but our knowledge of the embryogeny of genera from the tribe Cupressineae is distinctly limited. *Chamaecyparis* (5), *Thuja* (7), *Biota* (4), and only a few others have been investigated. Despite the uniformity of embryo type which usually exists among the members of a closely related group, two markedly different types have appeared among the investigated members of this tribe. It becomes of interest, therefore, to know into which of these groups uninvestigated members will fall or whether additional types will be discovered. These questions as they relate to *Cupressus arizonica* are given consideration in the present paper.

The morphology of the male gametophytes of this species was reported in an earlier work (6). Most of the material for the present study was taken from the same tree which furnished the material

for the previous investigation. Although *C. arizonica* is indigenous to the mountainous portions of Texas, it is not native in the region adjacent to College Station. The present findings, therefore, were checked against material obtained from trees grown at Alpine, Texas, where conditions closely approximate those under which *C. arizonica* grows naturally.

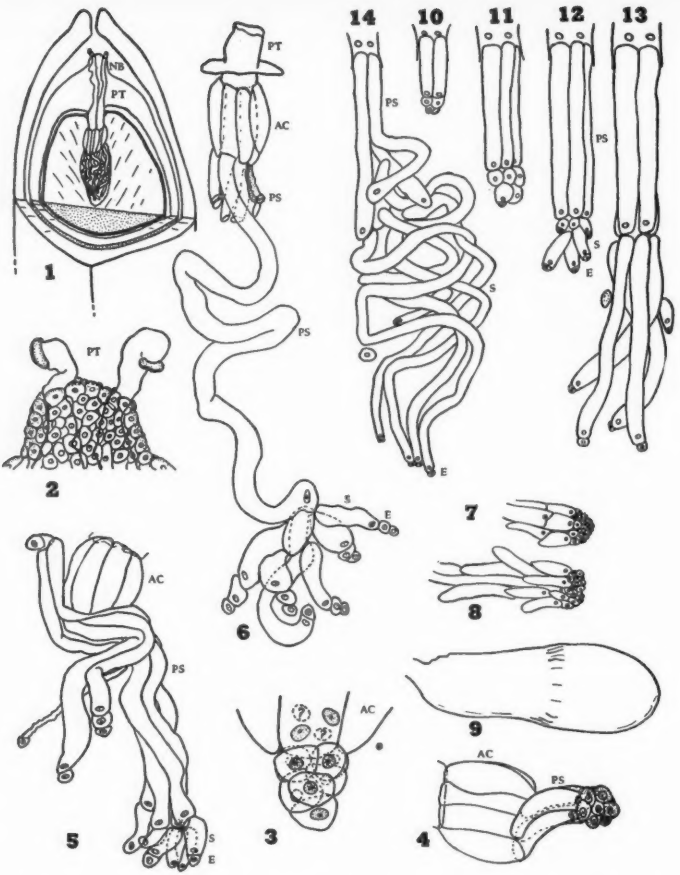
Material and methods

At College Station some plants produce both pollen cones and seed cones regularly, and still others none at all. In some trees there is a decided tendency toward monoecism, especially in young specimens of the second generation under domestication. Plants vary widely in the time at which the pollen is brought to maturity. Ovules with copious pollination droplets have been seen as early as January 30 and as late as March 15. Late spring frosts often kill some or all of the pollen cones. The plant from which most of the material was taken, however, has been a consistent producer of viable seed and has many offspring in this region. Seedlings vary widely in form and color, indicating considerable heterozygosity.

Parallel embryo studies were made in the summers of 1929, 1932, and 1936. Checks have been made against material from other trees in this region and from trees at Alpine. No significant differences were found from tree to tree or from season to season.

The ovules were opened with a razor blade. The nucelli with their inclosed female gametophytes were removed and dropped into 0.3 gm. molecular cane sugar. In this, as well as in the dissecting, staining, and mounting, the technique of BUCHHOLZ was followed. It was found possible to make camera lucida tracings of embryos freshly dissected and thus to obviate the necessity of mounting all the embryos.

Cupressus arizonica has at the apex of the nucellus a more or less prominent "nucellar beak" (figs. 1, 2) upon which the pollen grains germinate. The resulting tubes later lift the empty exines away from the surface of the beak, where they remain conspicuously elevated (figs. 1, 2). As a matter of routine, the nucellar beaks were examined for the number of their pollen tubes before the female gametophytes were removed from the nucelli.



FIGS. 1–14.—Fig. 1, semidiagrammatic drawing of sectioned upper portion of developing seed in midsummer of the first year. Fig. 2, detailed view of nucellar beak with protruding pollen tubes. Fig. 3, late proembryo; upper nuclei were overlain by archegonia and their number could not be definitely determined. Fig. 4, slightly older stage showing single embryo system with elongated prosuspensors. Fig. 5, still older stage showing two embryo systems; one of the units in that on the left did not elongate and one prosuspensor is undergoing collapse; embryo system on right is more nearly typical. Fig. 6, still older stage showing archegonial complex with two embryo systems from which seven of the eight prosuspensor units, together with their derivatives, have been omitted; prosuspensor elongation is complete and suspensors are beginning elongation. Figs. 7–9, functional (apical) embryos in winter rest condition; embryo shown in fig. 8 is undergoing vegetative budding. Figs. 10–14, stages in embryogeny of *Biota orientalis* as drawn by BUCHHOLZ and repeated here by permission. The steps are essentially the same as those found in *C. arizonica*. Figs. 13 and 14 represent stages beyond that shown in fig. 6. *ac*, archegonial complex; *e*, embryo; *nb*, nucellar "beak"; *ps*, prosuspensor; *pt*, pollen tube; *s*, suspensor.

It was found possible also to cut away the sides of the nucellus and to observe directly the number of tubes which had entered the archegonial chambers (figs. 1, 6).

In some cases the nucellar tissues which surround the upper reaches of the pollen tubes were dissected away and the irregular tubes exposed. An additional observation involved the counting of archegonia per archegonial complex.

Observations

As previously reported (**6**), *C. arizonica* develops erect urn-shaped ovules. Within these the nucellus is free from the integument, except at the base. It is protected except for the nucellar beak by a smooth tube-proof epidermis. The beak upon which the pollen germinates lies directly beneath a rather wide and shallow micropyle.

The pollen tube follows a tortuous course and often develops numerous male cells, which may fertilize numerous eggs within the same archegonial complex. Fertilization is accomplished about June 1, and two seasons are required for the ripening of the seed.

More than half the nucellar beaks examined received no pollen. Some pollen failed to germinate. A sample distribution of 300 pollen grains is summarized in table 1.

TABLE 1

Germinated grains on nucellar beak					Tubes in archegonial chamber				Tubes not arrived
Numbers.....	1	2	3	4	1	2	3	4	
Cases.....	262	34	3	1	196	7	1(?)	0	96

No case was found in which more than four grains had germinated on a single nucellar beak, and only once were three pollen tubes found entering a single archegonial cavity.

Some of the examinations were made before the tubes began to arrive at the archegonial chamber or before arrival was completed (some had not arrived in mid-July). For this reason a comparison of the number of tubes starting growth and finishing at the archegonial chamber has little significance.

Tubes in the same nucellus may arrive simultaneously or in succession. Some may not arrive at all.

The observed numbers of archegonia per complex ranged from one to thirteen. A single archegonium was observed but once, and its unusual size indicated abnormality. A sample distribution of archegonial numbers for 100 complexes counted at random is furnished in table 2.

Since a paper on multiple male cells (6) covered stages up to and including fertilization, a description of the proembryo logically falls next in order. The dissection technique, however, proved unsuited to the study of nuclear divisions and wall formation in the early proembryo. When dissected in the early stages of zygotic division,

TABLE 2

DISTRIBUTION OF NUMBER PER ARCHEGONIAL COMPLEX FOR 100 FEMALE GAMETOPHYTES

No. of archegonia	3	4	5	6	7	8	9	10	11	12	13	Total
Frequency	4	3	9	11	29	19	12	7	3	2	1	100

overlying archegonia and attached jacket cells obscure the early stages of the developing proembryo. In its later stages, however, the cells of its protruding tip may be seen below the apices of the archegonia. The elongation of certain cells to form a prosuspensor begins before the proembryo has reached the typical 16-celled stage so characteristic of pines and cedars. The even tiers of four cells are conspicuously absent (fig. 3). The early transformation of the cells homologous to the rosette tier of the pine proembryo into an elongated prosuspensor deprives the proembryo of *Cupressus* of its potential rosette layer, and in effect adds the cells of this layer to the suspensor system (figs. 4, 5). No rosette embryos were observed. Occasionally, however, a potential prosuspensor failed to make the usual elongation, and its attached embryo when crowded back gave the appearance of having arisen from the rosette (upper left, fig. 5).

In early stages the tubular units of the prosuspensor can be counted, and at this time their number is of value in determining the number of embryo systems (embryos from one zygote) in the entire

embryo complex (embryos from all zygotes). There is roughly one embryo system and of course one zygote for each group of four prosuspensor tubes (figs. 4, 5, 10–14). In my material the prosuspensors ranged in number from four to twenty, indicating from one to five zygotes. An embryo complex which shows twenty prosuspensor tubes cannot have resulted from less than five zygotes, and yet such a complex was observed in a female gametophyte to which there was attached but a single pollen tube. Numerous cases of three or more zygotes from a single tube were observed. Apparently either the multiple male cells from a single pollen tube effect multiple fertilizations or some of the embryos arise parthenogenetically. Since this species has been shown to have multiple male cells, the multiple fertilizations are more likely the correct explanation.

The elongation of the prosuspensor may or may not be followed immediately by the elongation of the true suspensor cells (figs. 5, 6).

Often the points of contact between the prosuspensor tubes and suspensor tubes are slight. In approximately 10 per cent of the cases they become detached and subsequently tangled beyond all hope of separation into distinct embryo systems.

In many cases vertical wall formation begins immediately following elongation of the prosuspensor, thus giving rise to a large number of loosely connected embryos with varying numbers of short suspensors and embryonal tubes (fig. 6). They may become completely separated from their connections, either by collapse and subsequent digestion of the connecting tubes, or by simple detachment of abutting tubes along their contiguous ends. In rare cases balloon-like distensions are formed.

A far more typical behavior is for the vertical wall formations, separations, and distensions just described to be postponed until after the elongation of the true suspensor cells (figs. 13, 14). At about this stage, which is reached approximately July 1, some of the single celled embryos temporarily cease dividing and go into a semi-thick walled condition similar to but less pronounced than that reported for *Biota* (4).

For most of the embryos development continues slowly through July, August, and a part of September, by which time all embryos

have been eliminated except the functional one. Of the dozen to five dozen embryos which may have arisen in a complex in the course of the summer, usually only four or five remain at the onset of the winter rest. Among these the functional one is usually clearly recognizable. This, the largest of the group, is crowded into the apex of the embryo cavity and there remains inactive during the winter period. At this time the embryos vary widely in their size and degree of development. Some show unmistakable sign of vegetative budding (fig. 8). Prominent embryonal tubes anchor the embryos firmly in place (fig. 8) among the partly digested cells of the female gametophyte. Although the embryos are, during the winter rest, rarely if ever in possession of tissue regions and cotyledonary primordia, they initiate these immediately upon resumption of active growth (figs. 7–9), and by the middle of April most of the embryos have reached full size.

It is clear that in all particulars, save the winter rest period, the development of the embryo of *C. arizonica* follows, to a remarkable degree, that already described by BUCHHOLZ for *Chamaecyparis obtusa* (**5**) and for *Biota* (**4**). In order to emphasize this fact, figures 10 to 14, which are copies of BUCHHOLZ's diagram of the embryogeny of *Biota*, are furnished herewith. They could as well have been drawn for *Cupressus*.

Biota is sometimes merged with *Thuja*. The former, as already pointed out, has extreme cleavage polyembryony. The latter has simple polyembryony and no cleavage. The evidence furnished by this report, therefore, can be considered to support the contention that the genus *Biota* of ENDLICHER is valid, and that from the evidence of its embryogeny, it lies closer to *Cupressus* and *Chamaecyparis* than to *Thuja*.

Summary

1. The steps in the early embryogeny of *Cupressus arizonica* are described.

2. The number of archegonia in a complex is found to range between 3 and 13.

3. More embryo systems were observed than could be accounted for on a basis of two fertilizations per pollen tube, indicating multiple fertilizations from multiple male cells.

4. The steps in the embryogeny, except for the speed of their passage and the insertion of a winter rest period, closely parallel those of *Biota orientalis*.

5. The resemblance of the embryogeny of *C. arizonica* to that of *Biota* is taken as further evidence that ENDLICHER'S genus *Biota* is valid, and that it more closely resembles *Cupressus* than *Thuja*, with which it is sometimes combined.

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RESPONSES OF BEAN AND TOMATO TO PHYTOMONAS TUMEFACIENS, P. TUMEFACIENS EXTRACTS β-INDOLEACETIC ACID, AND WOUNDING[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 480

GEORGE K. K. LINK, HAZEL W. WILCOX, AND ADELINE DES. LINK

(WITH TWENTY-TWO FIGURES)

Introduction

In our study of nutrition of tomato relative to crowngall development (23), we corroborated (24) BROWN and GARDNER'S findings (5) that *P. tumefaciens* (Smith and Town.) BERGEY *et al.*, incitant of crowngall, produces growth substances. Further, the crude extract was found to contain heteroauxin (24). To date scarcity of material has prevented analysis for other significant constituents. Application of the extracts and of heteroauxin was made with and without wounding to the bean and tomato. Wound responses of the bean in control experiments led to extended study of its reaction to puncture, incision, decapitation, and segmentation.

Because of recent excellent summaries on plant hormones and their effects, there is no need for a literature review in this paper. AVERY and BURKHOLDER'S translation and revision (3) of BOYSEN-JENSEN'S monograph and the second review by JOST (13) cite original and review papers. There is relatively little pertinent literature on *Phaseolus vulgaris*. European workers almost invariably use *P. multiflorus* (4, 10). WEHNELT (33) does not state what species of *Phaseolus* he used. SILBERSCHMIDT and KRAMER (30) used *P. vulgaris*. KÜSTER'S text cites the earlier literature on pathic tissues of this plant (17). BROWN and GARDNER (5), KRAUS, BROWN, and HAMNER (16), and HAMNER and KRAUS (9) have used *P. vulgaris*.

The voluminous literature on crowngall has been reviewed by RIKER and BERGE (28). Hence no attempt is made to treat it here.

[1] Supported in part by a grant from the Rockefeller Foundation to the University of Chicago. Additional cost of publication sustained by the writers.

Materials and methods

Experiments were performed from May 1936 through the following spring. The bean rather than the tomato was selected as chief test plant, since it lends itself more readily to greenhouse culture during Chicago winter conditions, develops rapidly with a massive seedling and later with large well defined members, and is less disposed to develop aerial roots under normal greenhouse conditions. The bean also produces nodules. The only troublesome infectious diseases were mildew, controlled by sulphuring, and blight, controlled by use of infection-free seed. *Phaseolus vulgaris* var. Red Kidney, although not quite so responsive as Golden Wax, was used because BROWN and GARDNER (**5**) and KRAUS and associates (**9, 16**) have used it. Tomato varieties Marglobe, Bonnie Best, and Ponderosa were used. No differences were noted in their behavior.

Day and night winter temperatures were approximately 70°–75° F. and 60° F. respectively. From March on, day temperatures tended to be higher (90° F.). Humidity in the houses was relatively low, Wardian chambers being used when high humidities were desired. Most plants were grown in soil under these conditions. The form of the plants changed as the season advanced, hypocotyl and internodes becoming shorter with increasing daylight. In addition beans were grown in the dark in quartz sand or soil at 80° F. and a relative humidity of 70 per cent. Beans grown in quartz sand in the light for prolonged periods were not so desirable for experimental purposes because of deficient growth. Some tomatoes grown in quartz sand received plus or minus nitrate nutrient solutions.

Since control plants wounded by puncture, incision, and transection made some responses similar to those obtained with extract, heteroauxin paste, or *P. tumefaciens*, experiments were made, primarily with bean, on the effects of wounding. Bacterial extracts on very young bean and apical tomato tissues caused killing. Second internodes of the bean were scraped and treated with 25 per cent acetic acid to determine whether later growth responses to the extracts were wound responses following local killing. Seedlings and young plants were punctured, incised, and/or segmented with or without removal of cotyledons (fig. 3). When severed, the portions were placed on moist sand flats in the dark. Observations were

made to ten days. Punctures were made with a needle to the center of the axis in young arched hypocotyls, and in older plants in upper and lower halves of the hypocotyl, first and second internodes. Vertical and transverse incisions were made in the same locations in the older plants, some of the latter with inserted glass or mica plates. Eight days old seedlings were incised transversely in the middle of the hypocotyl or decapitated directly below the cotyledons. Severed seedlings were similarly incised, also segmented at the middle of the first internode or at top or middle of the hypocotyls. Hypocotyls were left in one piece, halved, quartered, or further cut to minimum lengths of 1 cm.

The β-indoleacetic acid (heteroauxin) used was obtained in crystalline form from Merck. Preliminary experiments were made with 0.2 or 2 per cent heteroauxin in lanolin applied to tomato basal and apical internodes, some of the latter being decapitated. Later experiments were made using 3 per cent heteroauxin in lanolin, following BROWN and GARDNER (**5**) and KRAUS and associates (**9, 16**). Applications were made as before to tomato with and without puncture. Bean hypocotyls of different ages were inoculated at several sites with dosages estimated at 0.01 and 0.0005 cc., applied with a tuberculin syringe. Later, following LAIBACH'S method (**20**), concentrations were prepared ranging from 0.1165 to 0.0000122 per cent or $1:10^3$ to $1:10^7$, the initial concentration being LAIBACH'S 1:1 paste. Dilutions were made by weight in geometrical progression using lanolin water paste (1 part lanolin to 1 part water) as dilutant. In later experiments the 3 per cent lanolin paste was used as an additional concentration. Applications were made with and without wounding to various organs of the bean and less extensively to the tomato, unilaterally, by banding, or by covering decapitated organs. These tests were to determine the extent to which heteroauxin could induce the gamut of known pathic growth responses. With one exception, concentrations high enough to produce killing were not used. By decapitating epicotyls, blades of first leaves, and removing hypocotyls with and without applications of paste, experiments were made to determine whether foreign growth substance (heteroauxone, see discussion) can substitute for native growth substances (autoauxones, see discussion) of the bean or whether their augmenta-

tion, as in application of heteroauxin to the bean, would increase growth rate.

A strain of *P. tumefaciens* derived from a single cell isolation (W. M. Banfield) was used for direct inoculation and for preparation of all bacterial extracts. A 24 hours old culture grown on standard potato dextrose agar was applied with and without puncture to the upper and lower halves of the hypocotyl and the first and second internodes of the bean, apical and basal internodes of the tomato, and decapitated top internodes of both. An older culture grown in quantity in Kolle flasks was divided, one portion being extracted with ether, the residue taken up and applied, and the other killed with 1:10,000 merthiolate. Agar on which these organisms were cultured was applied to decapitated bean hypocotyls. The two broth media described later with 2 per cent agar added were similarly inoculated and used as a source of agar blocks in this experiment.

Galls incited by *P. tumefaciens* on tomato and control stem and leaf samples were extracted with acidulated chloroform according to THIMANN'S method (**32**). The residues were applied directly to the bean hypocotyl with and without puncture, and to second internodes. Gall extract only was applied to tomato seedlings, and all three to older tomatoes approximately 20 cm. tall.

In preparation of bacterial extracts, *P. tumefaciens* was cultured at 70° F. in two broth media, one containing 2 per cent dextrose and 0.1 per cent tryptophane in distilled water, the other containing in addition 1 per cent peptone. Heavy pellicles were produced in the latter. Ether extracts were made of cultures three weeks and three months old, the pellicles being extracted separately. The broth culture was shaken for several hours with an equal volume of anhydrous peroxide-free ether, then stored several days at 30° F., the ether decanted and evaporated. Pellicles, after removal from the medium by filtration through glass wool, were ground with it, and similarly treated with ether. All these preparations yielded viscous, noxious smelling, dark orange-brown residues which were stored at 50° F.

The extracts were applied directly (without mixture with lanolin) to the bean hypocotyl, first and second internodes with and without puncture, and to decapitated second internodes. First inter-

nodes of tomato seedlings and apical internodes of older plants were also treated. Applications were made to a limited number of *Avena* coleoptiles. Since the yield was very meager, a small blade hammered from a pin point was used to scrape the material from the evaporating dish and for its application to the plant. Minimum effective dosages were estimated at 0.0025 cc.

Preliminary identification of the presence of heteroauxin in the extracts was made by the hydrochloric acid, ferric chloride, amyl alcohol test specific for that substance (**1**, **15**). The standard consisted of a saturated (0.233 per cent) aqueous solution of β-indoleacetic acid (Merck) diluted in geometric series to give a final concentration of $1:10^4$. Volumes of 20 per cent hydrochloric acid and of 0.1 per cent ferric chloride equal to that of the test material were added (0.32 cc.), and gently boiled until the cherry red color of the lower dilutions or pink color characteristic of higher dilutions of heteroauxin was obtained. Amyl alcohol, one half (0.16 cc.) the volume of any other single ingredient, was added. With brisk shaking, the color passed into the alcohol.

Five mg. lots of extract were dissolved in ether, yielding a yellowish brown solution. After addition of the acid, the ether was driven off over a water bath and the test repeated as described, using the same volumes. In the course of boiling, the characteristic red or pink color appeared and later dissolved into the alcohol, this color becoming partially obscured by the yellow color of the initial solution.[2]

All samples for histological examination were killed and fixed in Navashin's solution.

Experiments and results

Inoculations with P. tumefaciens

Applied without wounding, *P. tumefaciens* produces no visible effects on the tomato and bean. Introduced by puncture or decapitation in the tomato, it causes galls, abnormally abundant adventitious roots, epinasty, stunting, and death (**25**) (fig. 1). In the bean, puncture of the axis leads to gall formation (figs. 2*A*, 14*G*, *I*).

[2] We are indebted to Professor F. C. Koch of the Department of Biochemistry for consultation and verification of results.

1

2

FIGS. 1, 2.—Fig. 1, *A*, *B*, moderately dry air; *C*, high humidity. *A*, control; *B* and *C* inoculated at *x* with *P. tumefaciens*. Note epinasty, gall, and adventitious roots in *B* and *C*. Tomato treated with heteroauxin or with extracts of *P. tumefaciens* produces identical gross effects (fig. 18). Fig. 2, *A*, bean; *B*, tomato; *C*, *Bryophyllum* internodes inoculated same date with *P. tumefaciens*. Galls of *A* and *B* have ceased growth; *C* still very active. All 6 weeks old, in moderately dry air.

Because of relatively low humidity of the room in which the plants were grown, inoculated decapitated stems did not develop the galls recorded by BROWN and GARDNER (5). Since the bean internode elongates rapidly and ceases to increase earlier than the tomato, bean galls do not attain the relative or absolute size of tomato galls (fig. 2*A*, *B*). *Bryophyllum*, being a slow but prolonged growing plant, produces galls which are still extending along the axis when tomato and bean galls of the same age have ceased growth (fig. 2*C*). Eventually huge galls are produced that kill the plant. In each of these plants the greatest response was provoked in the most actively growing area. The same area when older could fail entirely to respond, as in the bean hypocotyl.

WOUNDING

The injured bean, including all vegetative organs and the fruit (16), is highly disposed to tumor[3] formation, development of adventitious roots, and root regeneration (fig. 3). No data are available for floral parts. Punctures in the young internode produced local swelling (fig. 14 *F*, *H*). No enlargement occurred in the hypocotyl. Vertical incisions resulted in the usual callus formation. Transverse incisions with or without insertion of glass or mica plates caused local swelling above the wound, accompanied occasionally by roots, often fasciated, and/or marked callus formation. Similar responses occurred on basal ends of certain segments but rarely at the apical end of any segment. Apical segments of the hypocotyl and those 1 cm. in length at any level failed to respond. Longer segments showed more roots, more callus production, and less desiccation than shorter ones. Root production was usually but not always accompanied by swelling, occasionally by callus formation. In the first and second internodes, swelling occurred on incision and severing but root production was rare. Leaves severed just below the pulvinus from the petiole developed swelling at the cut.

These results indicate a definite polarization and concentration of root inciting substances. To check this hypothesis, 3 per cent

[3] Tumor is here used to designate any localized swelling due to anomalous growth (*i.e.*, a neoplastic swelling). Following KÜSTER, gall is used to designate a tumor incited by a parasite that derives its nutrients from the tumor tissues (17, 18). Hyperhydric tissues that produce swelling (including intumescences and surface callus), and internal callus that leads to swelling, are instances of tumors.

heteroauxin paste was applied at the apical end or at the middle of hypocotyl segments 4 cm. long. Roots appeared directly below the application.

In severed eight days old seedlings, removal of the cotyledons resulted in marked wilting, less basal swelling, fewer roots. Those produced were less well developed and often remained visible as pro-

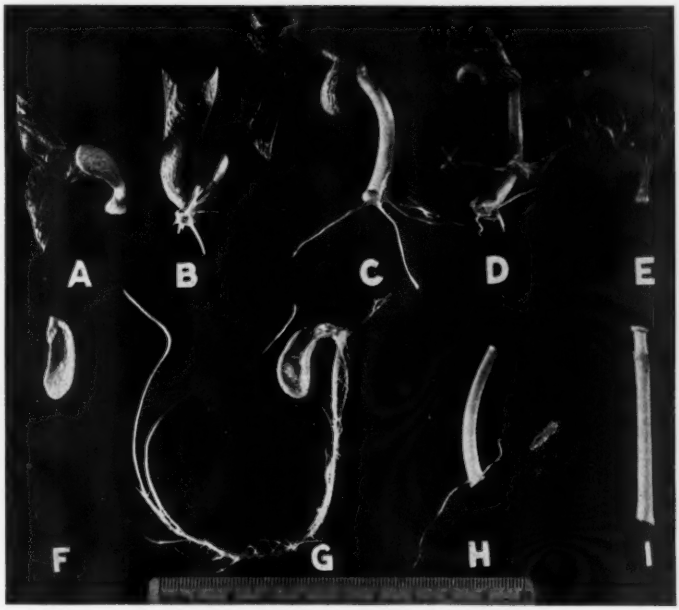


FIG. 3.—Segments of bean seedlings kept 10 days in wet sand flats in dark. *A*, severed immediately below cotyledons; swelling (internal callus) at basal end; whitening. *B*, like *A* but older; roots regenerated from swelling. Roots in all specimens in four rows, with tendency to fasciation. *C*, longer segment with swelling and longer roots. *D*, segment as *C* but incised at *x;* swelling and roots above upper surface of cut and at base. *E*, epicotyl with swelling (these occasionally develop roots). Severed leaves develop similar swellings. *F*, removed cotyledon with swelling. *G*, removed and heteroauxin treated cotyledon with large swelling and roots. *H*, segment of hypocotyl with swelling at base and top and roots at base. *I*, segment of hypocotyl and first internode decotyledonized, with swelling at base and large surface callus at top.

tuberances beneath the surface of the stem. In nine days old seedlings these differences were less marked, but of the same kind.

Development of axillary shoots following spontaneous blasting of the leaves of the embryonic epicotyl (fig. 4*D*) or experimental decapitation of the internodes (fig. 4*B*, *C*) indicates inhibiting effects of these and implies growth correlations dependent on polarization of the main streams of hormones moving from divergences toward and down the axis.

In the tomato, punctures gave slight swelling. Areas exposed by decapitation became covered by callus.

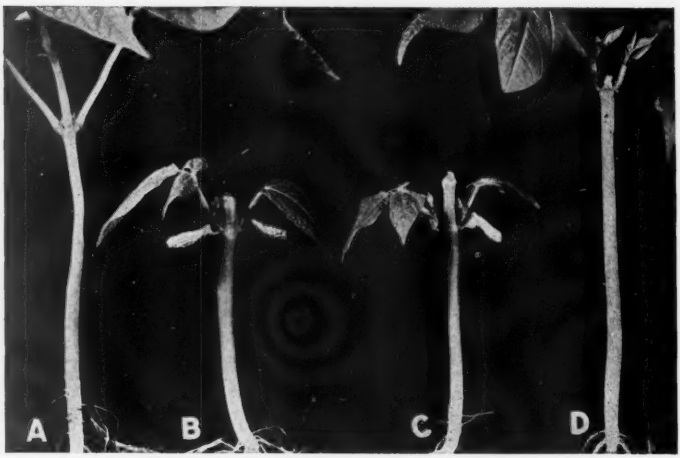


FIG. 4.—*A*, control; *B*, *C*, decapitated first internode. Note cotyledons still present, axillary shoots. Swelling of stump and hypocotyl due to heteroauxin paste 1: 10^3. *D*, seedling with spontaneous blasting of leaves of plumule. Note hypocotyl reaches second node of control, axillary cotyledonary shoots, and shriveled first internode.

β-INDOLEACETIC ACID APPLICATIONS

The demonstration that *P. tumefaciens* produces heteroauxin in culture (**24**) led to extensive bean and limited tomato experiments with the chemically pure substance.

ROOT.—Beans were germinated in soil and in clean quartz sand. To primary roots not over 7 cm. long in the former, 3 cm. long in the latter, as small a mass of paste as possible was applied, the site being indicated by *x* in the figures. It was found that the first 3 mm. of the root must be treated to get effects. The seedlings were then

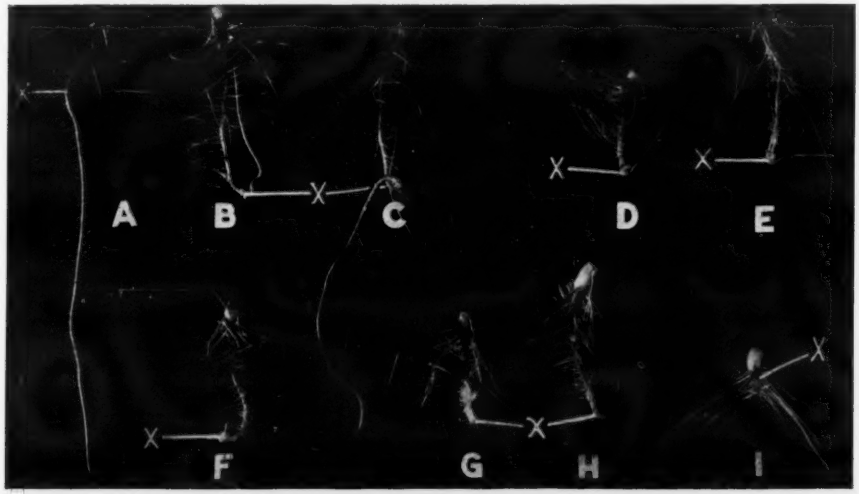


FIG. 5.—Primary roots, paste applied at *x*. *A*, *B*, *C*, *E*, *F*, *H*, *I*, 7 cm. long; *D*, *G*, 3 cm. long when treated. *I*, decapitated at *x*. *A*, water paste; *B–H*, heteroauxin paste; *B*, 5: 10^7, *C*, 2: 10^6, *D*, *E*, 1.2: 10^4, *F*, 2.5: 10^4, *G*, *H*, 3% paste. Note coil and swelling in *F*, *G*, *H;* swelling hyperhydric tissue breaking through epidermis; abnormally abundant laterals in all modified roots; resumption of growth, excepting at *G* and *H*.

laid on wet sand in flats, covered and kept in a dark room at 70°–80° F. and approximately 70 per cent humidity. Data were recorded after 24 and 72 hours. Photographs were made after five days (fig. 5). Sixteen plants were used for each dilution and for the controls. One set of roots (3 cm. long) was decapitated 4 mm. behind the tip.

The decapitated roots stopped elongation and promptly developed lateral roots in abundance (fig. 5*I*). The untreated and water paste roots grew vigorously (fig. 5*A*), the bend in the root being due to its contact with the wall of the flat. The controls had lengthened 30 mm. in 24 hours and 120 mm. in 72 hours (table 1, I).

The first macroscopically detectable effect was produced by concentration $5:10^7$. It produced positive right angle bending but no retardation (fig. 5*B*) was detectable by the technique used. Concentrations down to $2:10^6$ produced positive bending and retarded elongation for several days (fig. 5*B–H*). Later, growth was resumed at a normal rate. Concentrations below $4:10^6$ produced such sharp and repeated positive bending that in many cases coils resulted (fig. 5*C–F*) resembling snail shells. Even the coils resumed growth after several days. Concentrations 3 per cent, $1:10^3$, and $1:10^4$ stopped elongation completely in some plants and induced local swelling at the tip (fig. 5*G*, *H*). After a few days these semi-spindles developed great masses of glistening white hyperhydric tissues which welled out from below the epidermis. All roots showing a right angle bend or more serious disturbances prematurely developed lateral roots up to the zone of injury (fig. 5*B–H*).

These results, especially the local tumors and the coiling, suggested legume nodules and the search for heteroauxin or similar substances in cultures of *Rhizobium phaseoli* and of mycorrhizal fungi.

Hypocotyl.—All dilutions were applied to the intact hypocotyl. In dosages of 0.01 and 0.0005 cc. of 3 per cent heteroauxin paste, response was equal the first few hours, proportional to the dose thereafter. Approximately 0.01 cc. 3 per cent heteroauxin paste applied unilaterally to hypocotyls before straightening of the arch produced negative curvature within a few hours; at times curvatures as great as 60° were noted (fig. 6*B*). Responses were most vigorous during the night. The next gross response was temporary retarda-

TABLE 1

CHANGE IN ELONGATION (IN MILLIMETERS) DUE TO HETEROAUXIN PASTE APPLIED

ORGAN	CONTROL*	WATER† PASTE	3%	1:10³	5:10⁴	2.5:10⁴	1.2:10⁴	6:10⁵	3:10⁵	1.5:10⁵	7:10⁶	4:10⁶	2:10⁶	1:10⁶	5:10⁷	2.5:10⁷	1:10⁷	AVERAGE‡
	I. Unilaterally to primary root tip																	
Root after 24 hrs.	21	28	0	0	0	0	0	0	0	0	0	22	20	25	26	28	29	25
Root after 48 hrs.	56	63	27	32	50	28	44	44	52	58	59	64	68	60	60	69	61	51
	II. Unilaterally to hypocotyl																	
Hypocotyl	.....	19	37	32	36	22	34	22	25	29	25	21	24	21	20	26	22	26
1st internode	.....	54	45	48	47	56	53	36	50	51	45	57	61	54	64	82	58	53
	III. By banding hypocotyl a. Green in light																	
Hypocotyl	.....	15	36	25	26	28	38	35	35	30	31	22	§	.....	.....	.....	.....	30
1st internode	.....	45	42	38	33	34	55	40	31	39	47	36	.....	.....	.....	.....	.....	39

* Control means plants not altered experimentally. Values based on averages of at least 24 plants.

† Plants treated with water paste (1 part lanolin plus 1 part water) applied as indicated in the titles. Values based on only four plants for each experiment in V, VI, XI–XVI.

‡ Average means average change in elongation of organs in heteroauxin paste treated plants. If computed only on basis of usually effective concentrations (3%–4:10⁶), the values for differences between water paste and heteroauxin paste treated plants would be more striking. Since only four plants were used for each concentration in experiments V, VI, XI–XVI, the numerical values for particular concentrations cannot be given much quantitative weight. The organs of the bean being highly variable in length, repetition of the experiments using statistically significant numbers is desirable. While readings and measurements were made and recorded daily for a week, only the differences between initial and final measurements are given here. In many instances second and third day measurements are the most striking. The experiments extended from October to May, hence variations in lengths of organs in different experiments.

§ Concentration not tested.

TABLE 1—*Continued*

Organ	Control*	Water† paste	3%	1:10²	5:10⁴	2.5:10⁴	1.2:10⁴	6:10⁵	3:10⁵	1.5:10⁵	7:10⁶	4:10⁶	2:10⁶	1:10⁶	5:10⁷	2.5:10⁷	1:10⁷	Average‡
	III. By banding hypocotyl—*Continued* b. Etiolated in dark																	
Hypocotyl		160		80														
	IV. To decapitated hypocotyl a. Green in light 1st experiment																	
Hypocotyl		4.8	7	4	6	5	2	4	9	2	4	Lost	4	4	4	3	4	4.4
	2nd experiment																	
Hypocotyl		8.3	12	1	13	12	11	13	11	12	7	17						10
	b. Etiolated in dark																	
Hypocotyl		5		9														
	V. To wounds of decotyledonized plants																	
Hypocotyl	10	14	22	20	26	15	21	16	19	9	23	14	10	7	17	13	16	16
1st internode	33	33	17	12	19	23	15	30	32	34	26	34	32	45	31	28	33	27
2nd internode	5	4	0	2	3	6	1	2	3	4	3	5	5	6	4	4	5	3

TABLE 1—*Continued*

Organ	Control*	Water† paste	3%	1:10²	5:10⁴	2.5:10⁴	1.2:10⁴	6:10⁵	3:10⁵	1.5:10⁵	7:10⁶	4:10⁶	2:10⁶	1:10⁶	5:10⁷	2.5:10⁷	1:10⁷	Average‡
VI. To cotyledons on plants																		
Hypocotyl	31	17	25	29	34	27	26	27	21	24	20	20	15	20	25	28	29	25
1st internode	66	61	33	52	37	58	50	63	77	63	59	56	62	58	56	57	60	62
2nd internode	20	17	13	16	23	21	27	30	32	25	14	21	19	27	19	18	16	21
VII. To decapitated epicotyl 1st experiment																		
Hypocotyl	9	.....	.....	18	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1st internode	25	.....	.....	5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
2nd experiment																		
Hypocotyl	32	32	32	18	17	22	19	24	20	20	30	26	30	26	27	23	32	24
1st internode	8	8	8	9	8	13	12	17	8	8	9	8	11	10	7	10	10	10
VIII. By banding first internode																		
Hypocotyl	.....	6	10	13	15	4	13	9	4	5	9	13	18	9	7	3	.....	8
1st internode	.....	36	30	23	30	22	32	39	30	35	36	31	49	36	35	34	.....	32.4
IX. Unilaterally to first internode																		
Hypocotyl	.....	5	8	15	17	8	12	4	11	15	2	9	9	10	Lost	3	Lost	9.4
1st internode	.....	40	35	39	38	34	32	36	37	33	29	34	40	28	Lost	31	Lost	35

TABLE 1—*Continued*

ORGAN	CONTROL*	WATER† PASTE	3%	1:10³	5:10⁴	2.5:10⁴	1.2:10⁴	6:10⁵	3:10⁵	1.5:10⁵	7:10⁶	4:10⁶	2:10⁶	1:10⁶	5:10⁷	2.5:10⁷	1:10⁷	AVERAGE‡
X. To decapitated epicotyl of decotyledonized plant																		
Hypocotyl	.....	− 3	− 2	4	5	9	2	− .5	− 2	3	2	− 1	0	− 2	2	7	1	2
1st internode	.....	6	5	6	6	5	10	7	5	7	5	5	6	11	6	6	7	6
XI. To decapitated petioles of second node																		
Hypocotyl	33	34	21	22	23	25	29	26	22	25	33	34	33	28	34	42	30	28
1st internode	32	28	35	40	38	30	45	42	34	37	31	35	25	28	31	28	27	34
2nd internode	13	14	16	28	12	10	21	18	18	14	11	15	23	8	12	13	13	15
Petioles	9	7	5	9	8	8	7	6	6	6	7	7	7	6	8	7	5	7
XII. To upper side of one petiole of second node																		
Hypocotyl	24	.....	27	20	29	27	22	16	25	17	19	18	19	.....	.....	.....	.....	22
1st internode	69	.....	43	62	53	58	69	55	65	67	65	66	36	.....	.....	.....	.....	58
2nd internode	11	.....	20	15	32	16	13	13	12	15	16	16	10	.....	.....	.....	.....	16
Untreated petiole	43	.....	51	49	49	46	53	49	50	49	45	43	38	.....	.....	.....	.....	47
Treated petiole	43	.....	28	45	40	40	47	39	50	50	45	44	45	.....	.....	.....	.....	43
XIII. To lower side of one petiole of second node																		
Hypocotyl	24	.....	32	20	17	22	32	24	25	9	30	42	23	.....	.....	.....	.....	34
1st internode	69	.....	37	47	57	61	59	61	55	43	46	60	61	.....	.....	.....	.....	57
2nd internode	11	.....	8	13	23	16	11	12	11	25	18	20	26	.....	.....	.....	.....	16
Untreated petiole	43	.....	37	43	57	53	54	47	55	47	45	38	43	.....	.....	.....	.....	46
Treated petiole	43	.....	17	31	47	35	43	43	50	43	38	41	45	.....	.....	.....	.....	50

TABLE 1—*Continued*

Organ	Control*	Water† paste	3%	1:10²	5:10⁴	2.5:10⁴	1.2:10⁴	6:10⁵	3:10⁵	1.5:10⁵	7:10⁶	4:10⁶	2:10⁶	1:10⁶	5:10⁷	2.5:10⁷	1:10⁷	Average‡
	XIV. By banding one petiole of second node																	
Hypocotyl	24	.....	38	22	17	17	19	17	27	33	21	20	15	.....	.....	.....	.....	23
1st internode	69	.....	41	33	62	45	48	47	55	70	53	59	50	.....	.....	.....	.....	52
2nd internode	11	.....	7	11	12	38	16	20	20	15	22	22	22	.....	.....	.....	.....	16
Untreated petiole	43	.....	41	45	52	51	45	57	50	48	43	43	47	.....	.....	.....	.....	47
Treated petiole	43	.....	22	17	30	41	38	43	44	50	48	47	53	.....	.....	.....	.....	39
	XV. To upper side of one leaf of second node																	
Hypocotyl	24	.....	7	37	19	19	18	15	17	21	21	24	17	.....	.....	.....	.....	20
1st internode	69	.....	35	58	72	63	63	49	69	60	59	49	53	.....	.....	.....	.....	57
2nd internode	11	.....	6	15	22	22	11	26	26	16	18	14	16	.....	.....	.....	.....	17
Petiole untreated leaf	43	.....	48	45	60	50	52	.....	57	.....	.....	.....	.....	.....	.....	.....	.....	52
Petiole treated leaf	43	.....	32	45	57	50	50	56	47	50	50	43	42	.....	.....	.....	.....	47
	XVI. To lower side of one leaf of second node																	
Hypocotyl	24	.....	25	20	29	17	39	15	17	22	20	15	17	.....	.....	.....	.....	22
1st internode	69	.....	25	45	53	45	48	49	49	60	47	45	53	.....	.....	.....	.....	47
2nd internode	11	.....	3	15	32	18	16	26	9	16	3	20	16	.....	.....	.....	.....	15
Petiole untreated leaf	43	.....	35	51	.....	.....	.....	.....	50	.....	33	.....	.....	.....	.....	.....	.....	42
Petiole treated leaf	43	.....	16	40	49	51	45	50	47	52	33	53	49	.....	.....	.....	.....	44

tion of hypocotyl elongation and of epicotyl unfolding (fig. 6*B*), although in time the treated plants attained hypocotyl length greater than normal. Whitening at the site of application appeared in one to two hours, depending upon the concentration and amount of paste used. After several hours definite swelling developed at and below the site of application (fig. 6*B*), extending downward below the soil line. (When after the application of 3 per cent heteroauxin paste to the hypocotyl, incisions were made and glass plates in-

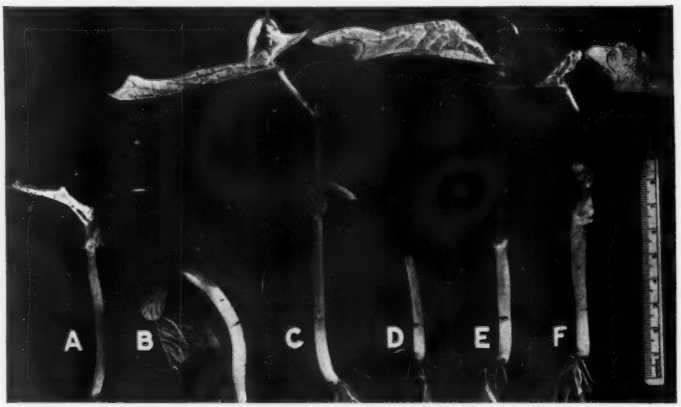


FIG. 6.—*A*, control; *B*, seedling treated unilaterally with 1: 10^3 heteroauxin paste; photographed after 6 hours. Note negative bending, retardation of elongation and of unfolding of epicotyl, swelling of hypocotyl greatest at and below site of application. Black line in each specimen indicates soil line. *C*, control; *D*, decapitated hypocotyl with water paste; *E*, decapitated hypocotyl with 1: 10^3 heteroauxin paste. Note local swelling, whitening, and general diameter increase. *F*, hypocotyl banding with 1: 10^3 paste. Note diameter increase of hypocotyl and first internode, lengthening of hypocotyl and shortening of first internode.

serted, directly below the application at hourly intervals effects were found to have passed below that point in two hours or less.) Later the hypocotyl also swelled along all radii, from a short distance above the site of application to the soil line. The average increase in diameter was 20 per cent. If application was made near the cotyledons, the first internode also swelled. Finally the site of application was marked by a colorless intumescence which projected above the level of general swelling (fig. 7*A*). The new tissues were

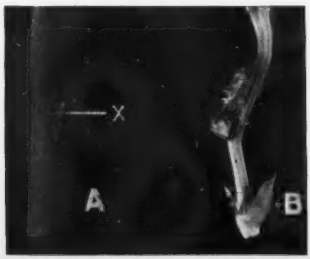
pathic. This was evident in the observation that during fumigation with hydrocyanic gas they were killed while the non-injured tissues were not affected. In moist air these tissues molded and in dry air they desiccated more rapidly than did the non-treated tissues.

The treated region developed abundant adventitious roots which did not emerge unless the air was quite moist (fig. 8*C*, *D*). No difference was found in the number of roots produced in reference to point of application on the hypocotyl circumference, whether on a line under or between the cotyledons.

Paste was also applied by banding the hypocotyl just below the arch (figs. 6*F*; 8*B*, *E*; 9*A*). Usually this did not produce bending, but was followed by more pronounced expression of the symptoms noted for unilateral applications. Generally banded hypocotyls attained greater length than controls (figs. 6*C*, *F*; 8*A*, *B*); at times the higher concentrations, especially 3 per cent, repressed elongation. Banding of the hypocotyl shortened the first internode (figs. 6*C*, *F*; 8*A*, *B*). The highest dilution effective for the hypocotyl was $4:10^6$ irrespective of mode of application. While the treatment increased diameter as well as later elongation, the final length of the hypocotyl usually was about that of a normal plant (table 1, II, III).

Age of the area to which heteroauxin was applied was significant. When only 1 cm. long, or directly below the cotyledons while still arched, the hypocotyl was unresponsive. Hypocotyls ten days old were markedly less reactive than those eight days old. When 15 days old they gave no response unless wounded. That this is not due to loss of reactive capacity by the cells but presumably to increased impermeability of the aged epidermis was demonstrated by applications of 3 per cent paste to old hypocotyls or internodes incised longitudinally. In moderately dry air these developed massive calluses full of root primordia. Placed in moist air, enormous root development took place (fig. 9*B*). In a few plants secondary tumors as well as roots apparently developed distally to the site of application. Great care had been taken to prevent spread of the inoculum. Freehand sections revealed continuity between the main and the smaller swellings. Frequently roots arising near the site of injury elongate downward just below the epidermis, without emerging.

DECAPITATED HYPOCOTYLS.—Decapitated hypocotyls develop surface callus tardily. If still young at the time of transection they elongate for a few days and then contract. This shortening was



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FIGS. 7, 8.—Fig. 7, *A*, hypocotyl 8 days after unilateral treatment with 1: 10^3 paste. *x* is site of sections of figs. 21 and 22. Smaller amounts of paste produced smaller involved areas. Sections of figs. 21 and 22 prepared from hypocotyl treated with much smaller amounts. *B*, second internode treated with 1: 10^3 paste, kept in moist air. Note roots. Fig. 8, *A*, control; *B*, hypocotyl banded with 1: 10^3 paste; kept in moderately dry air. Note greater thickening and elongation. *C* and *D* kept in very moist air. *C*, control; *D*, hypocotyl treated unilaterally with 1: 10^3 paste. Note roots at and below site of treatment. *E*, *F*, etiolated plants. *F*, control; *E*, hypocotyl banded with 1: 10^3 paste. Note bending, swelling, decreased elongation, retardation of epicotyl development, abundant roots in four rows.

noted in all hypocotyls measured. Treatment of the surface with control paste did not affect the elongation but led to more callus development. Heteroauxin paste led to increased elongation, to diameter increase, to swelling at the tip, and to root formation (fig. 6*D*, *E*; table 1, IV). Concentrations down to $7:10^6$ produced general diameter increase and local swelling. Concentrations below $2.5:10^4$ produced whitening below the site of application as well as initial constriction immediately below the treated surface. Roots were produced by concentrations of $7:10^6$ and greater. They were sparingly produced in dry but abundantly in moist air, developing in and below the tumors.

ETIOLATED HYPOCOTYL.—Unilateral and band applications to the etiolated seedlings in concentrations 3 per cent and $1:10^3$ definitely retarded elongation (fig. 8*E*, *F*). No other dilutions were tried. Applications to the decapitated hypocotyl definitely increased elongation over that of the decapitated control, which elongated very slightly. Unilateral applications were followed by negative bending; banding applications by no bending. The tumors produced attained large size, 100 per cent increase over the normal hypocotyl diameter not being exceptional. Band applications at any level of the hypocotyl led to development of four rows of roots below the site of application down to the soil level (fig. 8*E*). If the first internode was banded, roots developed below the band on the first internode and on the hypocotyl (fig. 9*A*). Unilateral applications led to root development at the site of application and on the side immediately below (table 1, III*a*, IV*b*).

SEGMENTS OF HYPOCOTYL.—Treated segments of the hypocotyl showed whitening, swelling, root and callus production at the surfaces, and apical as well as basal roots. The only concentration used was 3 per cent.

DECOTYLEDONIZED SEEDLING.—Applications of paste to removed cotyledon sites in dilutions up to $4:10^6$ led to diameter increase of the hypocotyl. Unfolding of the epicotyl was retarded by dilutions up to $6:10^5$. This treatment increased elongation relative to decotyledonized water paste plants (fig. 10*C*, *D*) but this increase did not equal that of the normal plant (fig. 10*A*, *B*, *E*; table 1, V).

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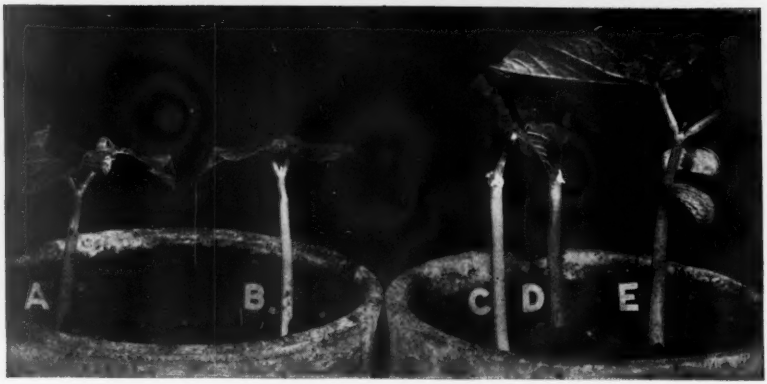

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FIGS. 9, 10.—Fig. 9, *A*, etiolated plants. To left, epicotyl banded with 1: 10^3 paste. (This experiment first performed by Dr. JOHN W. MITCHELL of this department.) Note swelling and retarded elongation of first internode, roots at site of tumor and in four rows throughout entire length of hypocotyl. (Tops of plants bent by bell jars.) *B*, plants grown in moderately dry air; hypocotyl and first internodes ceased elongation. Upon unilateral application of 3% paste no gross responses occurred. Longitudinal incision of hypocotyl and first internode and application of 3% paste there resulted in whitening, in an enormous callus which lengthened and widened the slit, and in roots mostly in four rows (if plants were kept in moist air). Roots established themselves in soil and became functional. In drier air, roots did not emerge. Fig 10 *E*, control plant. *A*, *B*, decotyledonized plants; *C*, *D*, decotyledonized and wounds treated with 5: 10^4 paste. Retarded development in *A* and *B* but increase in *C* and *D;* greater diameter increase in *C* and *D*. Cotyledons removed with razor blade. For paste treated cotyledons, see fig. 3*G*.

SEVERED COTYLEDONS.—Since cotyledons occasionally develop roots after removal from the plant, the cotyledons of the previous experiments were kept in pairs, one of each pair being treated with the paste. They were then placed on moist sand in the dark. Cotyledons pulled from the plants are more likely to develop callus and roots than those cut from the plant. The former procedure probably removes more of the active part of the cotyledon from the axis. Of 30 non-treated cotyledons, only two developed roots (fig. 3*F*) while 14 of 30 so treated developed roots (fig. 3*G*). Concentrations 3 per cent and $1:10^3$ to $1.5:10^5$ were effective. All roots were vigorous excepting those produced by concentration $1.5:10^5$. Many cotyledons did not develop roots until eight or nine days after removal and treatment.

TREATED COTYLEDONS.—Cotyledons on young plants were treated with pastes of all concentrations. On the average, treatment increased the length of both the hypocotyl and of the first internode and delayed abscission of cotyledons (fig. 11; table 1, VI).

DECOTYLEDONIZED AND DECAPITATED FIRST INTERNODE.—Treatment with pastes of wounds resulting from these operations led to general diameter increase (10 per cent) of the first internode and hypocotyl in dilutions up to $4:10^6$. In the stronger concentrations, local swelling at the cut (20–40 per cent) as well as massive surface callus developed. Whitening developed immediately below the site of application in the stronger concentrations (fig. 12*A–C*). On the average, treatment increased elongation of the hypocotyl (table 1, X).

UNILATERAL APPLICATION TO, AND BANDING OF, FIRST, SECOND, AND THIRD INTERNODES.—Unilateral application of the pastes to the internodes led to bending, retardation of elongation, and local swelling in dilutions up to $4:10^6$ (figs. 13*A*, *D;* 14*D*). The hypocotyl was lengthened. Concentrations 3 per cent and $1:10^3$ produced whitening (fig. 13*A*, *B*). Roots developed from the tumors (fig. 7*B*). Banding of first internode led to temporary retardation, then greater ultimate elongation of the hypocotyl and shortening of the first internode. The end point of concentrations was the same as in unilateral application. Other effects were identical except bending (table 1, VIII, IX).

DECAPITATED FIRST INTERNODE.—Treatment of decapitated first internodes decreased hypocotyl elongation but increased diameter increase (fig. 12*D*, *E*). The end point of reaction was dilution $7:10^6$ (table 1, VII).

DECAPITATED SECOND AND THIRD INTERNODES.—Decapitation of these internodes was not followed by elongation. Under moist conditions a surface callus developed (fig. 13*H*). Under the usual condi-

FIG. 11.—*A*, *B*, cotyledons treated with $5:10^7$ paste; *C*, *D*, *E*, control plants. Note increased elongation of hypocotyl and retention of cotyledons in *A*, *B*. *A* and *B* show method of measurement: a gardener's label was placed on the pot and measurement of hypocotyl was made from top of label. Most controls used during May appeared like *C*, *D*, and *E*.

tions prevailing during the experiments no surface callus developed (fig. 13*I*). Application of pastes up to dilutions $4:10^6$ produced local swelling and surface callus (fig. 13*E–G*). General diameter increase ranging from 10 to 20 per cent was produced by 3 per cent and $1:10^3$ paste. Roots developed abundantly from the surface callus and the swelling (fig. 13*E*), especially in moist air.

SECOND NODE.—Young petioles severed immediately below the blade pulvinus were treated with the pastes (fig. 12*F–H*). In dilu-

tions up to $4:10^6$ the petiole increased in diameter, swelled locally at the cut, and developed surface callus. Length of the hypocotyl was increased over that of water paste treated plants (fig. 12*H*)

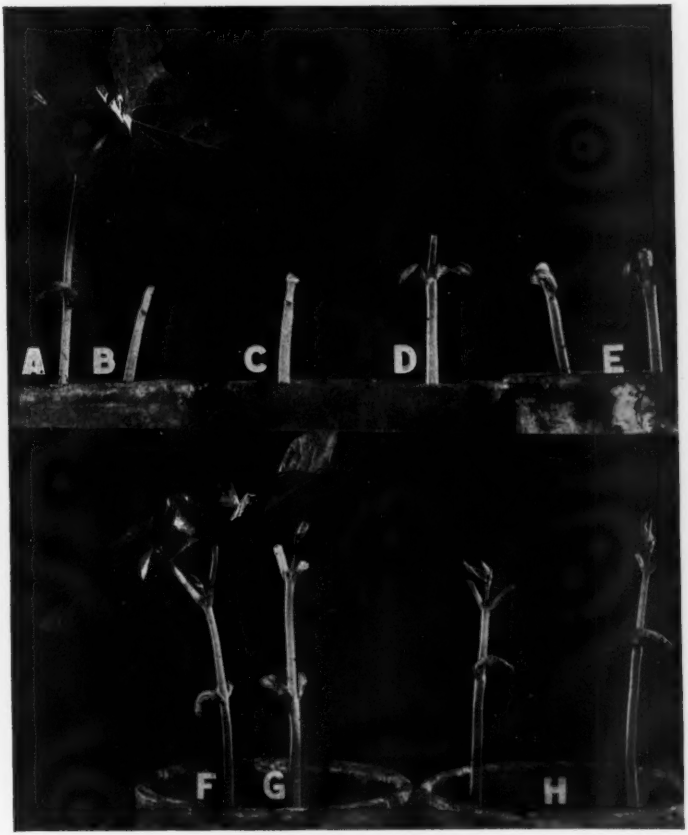
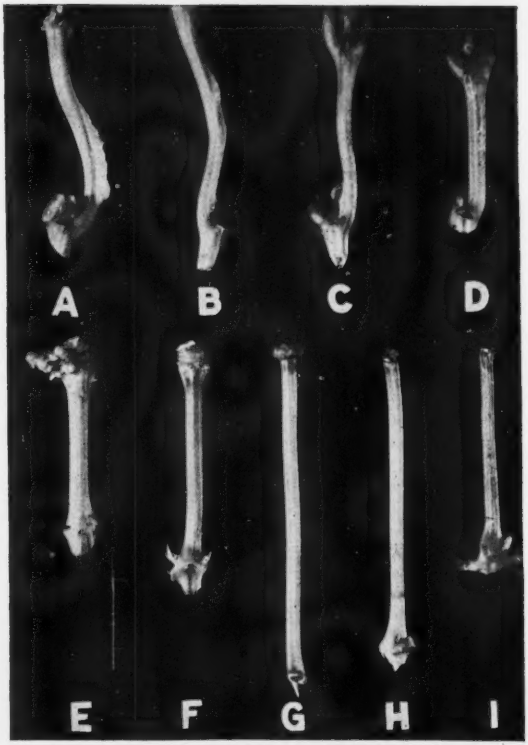


FIG. 12.—*A*, control; *B*, decotyledonized plant with decapitated first internode. *C*, same treated with $3:10^5$ paste. Note local swelling of first internode and general thickening of hypocotyl. *D*, decapitated first internode. Note increase in length and of diameter of hypocotyl. See fig. 4*D* for nature's experiment. *E*, decapitated first internode treated with $1:10^3$ paste. Note diminished elongation of hypocotyl, first internode, as well as general diameter increase and local swelling due to concentrated paste. *F*, control; *H*, decapitated petioles; *G*, same treated with $1:10^3$ paste. Decapitation of petioles resulted in increased length of hypocotyl and of second internode. Application of paste shortened hypocotyl, lengthened first and second internodes.



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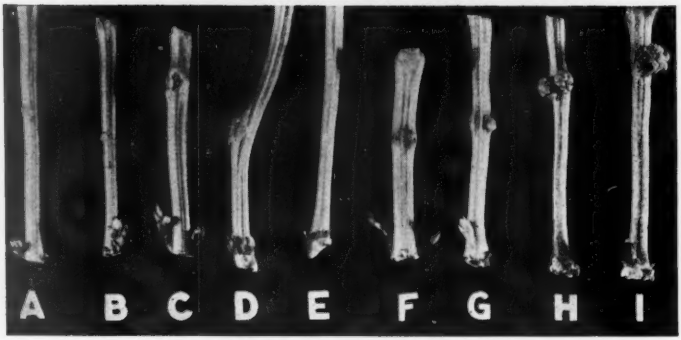

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FIGS. 13, 14.—Fig. 13, *A–C*, second internodes treated with paste. *A*, 3%; *B*, 1: 10^3; *C*, 4: 10^6; *D*, water paste; *E–I*, decapitated second internodes. *E*, treated with 3% paste; *F*, with 1: 10^3; *G*, with 7: 10^6; *H*, *I*, with water paste. Note surface callus in *E–I*, local swelling, general diameter increase and roots in *E* and *F*. Fig. 14, treated second internodes. *A*, water paste; *B*, ether extract of non-inoculated dextrose-tryptophane medium; *C*, ether extract of dextrose-tryptophane culture of *P. tumefaciens* (taken after internode had straightened after initial bending). Note central sunken area due not to killing but to locally inhibited growth. *D*, 1: 10^3 paste; *E*, *P. tumefaciens* applied without puncture; *F*, needle puncture; *G*, 2 weeks old gall following inoculation of *P. tumefaciens* by puncture; *H*, 8 weeks old callus at puncture site; *I*, 8 weeks old gall at puncture site.

whose hypocotyl was longer than that of controls (fig. 12*F*). Length of the first and second internodes was increased by petiole decapitation (fig. 12*H*). These results indicate inhibiting effects of leaves on elongation of the axis below, and of the growing region above them. Treatment with paste further increased differences in lengths of hypocotyls and second internodes (table 1, XI).

APPLICATION TO UPPER AND LOWER SIDES AND BANDING OF PETIOLES OF SECOND AND OTHER NODES.—Dilutions up to $7:10^6$ produced negative bending as well as local swelling. Higher concentrations also produced whitening (fig. 15*A*, *C*). Application of paste to the upper and lower sides of petioles led to slight lengthening of the hypocotyl, first and second internodes. Treatment of upper side led to shortening, while treatment of lower side led to lengthening of the petiole.

Banding of the petiole led to local swelling, to assumption of the vertical position by the petiole, and to decreased elongation. Concentration $4:10^6$ was the end point. The higher concentrations produced whitening (fig. 15*B*; table 1, XII, XIII, XIV).

PULVINI AT BASE OF LEAF BLADE.—Treatment of either the upper or lower side of the pulvini of leaves of the second node resulted in negative bending (fig. 16). Concentrations $7:10^6$ and greater were effective. In treatment of the lower side of the pulvinus higher concentrations turned the leaves completely back so that the lower surface became the upper (fig. 16*A*, *C*, *D* righthand). Treatment of the upper side with high concentrations turned the leaves downward past the vertical position (fig. 16*D*, lefthand).

LEAF BLADES.—Leaves of the second node were treated by application of strips of paste across the middle of the leaves from margin to midrib or across the entire width on the lower or upper side. In young leaves dilutions up to $7:10^6$ and in older leaves up to $1.5:10^5$ diminished elongation of the petiole (fig. 15*F*); treatment of the lower side increased elongation (fig. 15*E;* table 1, XV, XVI). In all cases treatment caused upward folding along the midrib (fig. 17*A*, *B*). In concentrations above $6:10^5$ torsions of the petioles developed in leaves treated only on one-half the blade. The treated half of the leaf was turned up vertically or even to a new horizontal position with the lower side uppermost.

In younger leaves, that is, leaflets of the third node (fig. 17*C–E*), the effects were essentially the same with additional symptoms.

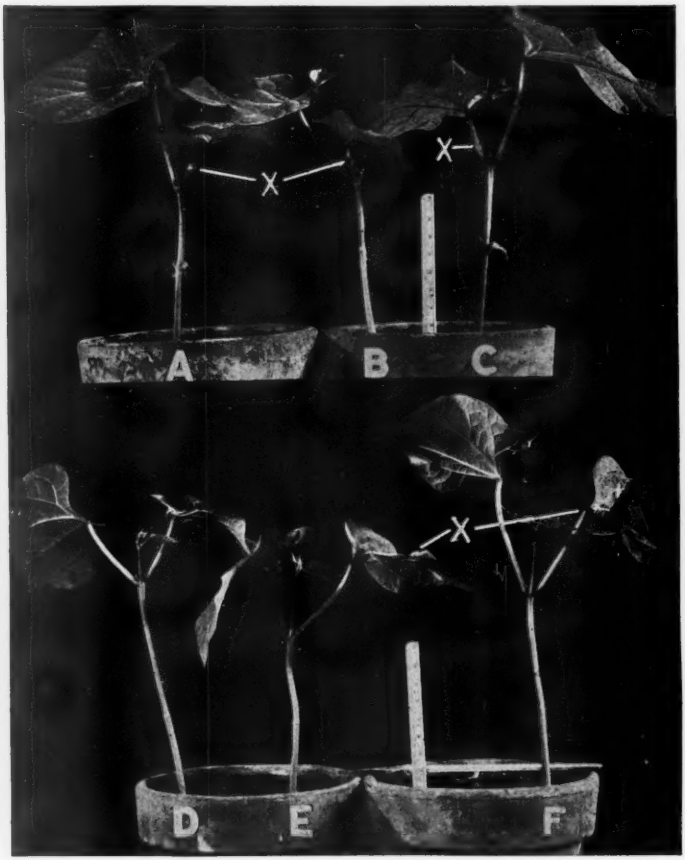


FIG. 15.—*A–C*, petioles treated with paste. *A*, petiole treated with 2.5: 10^4 paste on upper side at *x*; *B*, treated at lower side at *x*; *C*, banded at *x*; *D*, control; *E–F*, leaves treated; *E*, leaf on right hand treated on lower side. Note elongated petiole, *x*. *F*, leaf on right hand treated on upper side, petiole shortened, *x*.

The form of such leaves was affected. At and beyond the site of application the leaf was less expanded than normally or there was

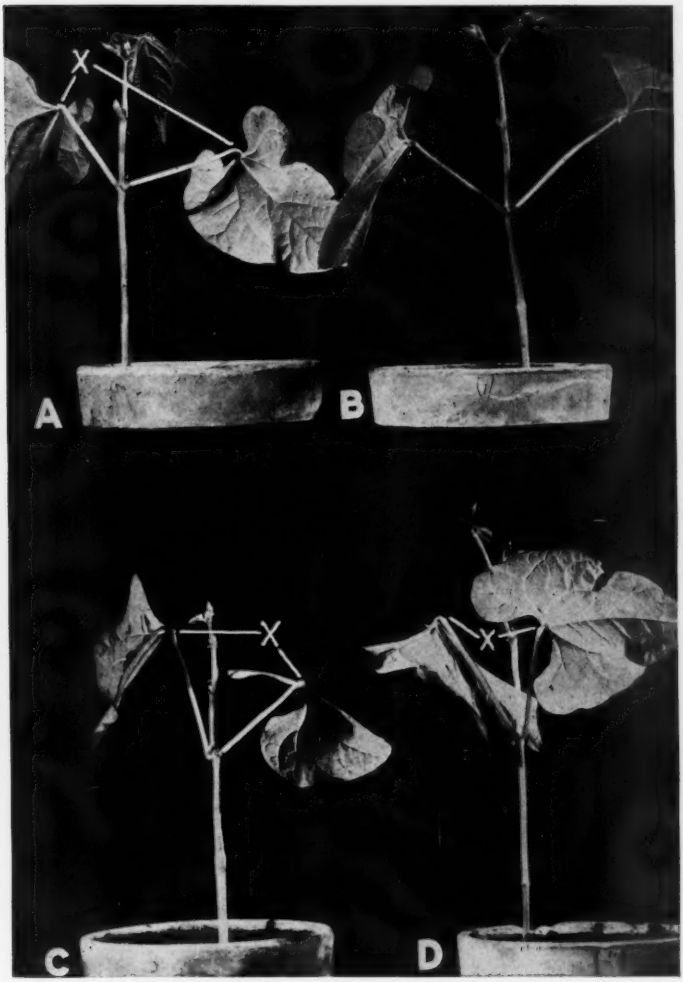


FIG. 16.—*A*, *C*, *D*, pulvini of lefthand leaves treated on upper side at *x* with heteroauxin paste; pulvini of righthand leaves treated on lower side at *x*. *A*, 1.2: 10^4; *C*, 1: 10^3; *D*, 3% paste. *B*, pulvini treated with water paste. Note that lower sides of leaves in *A*, *C*, *D*, right, have become the upper sides due to torsions in petioles.

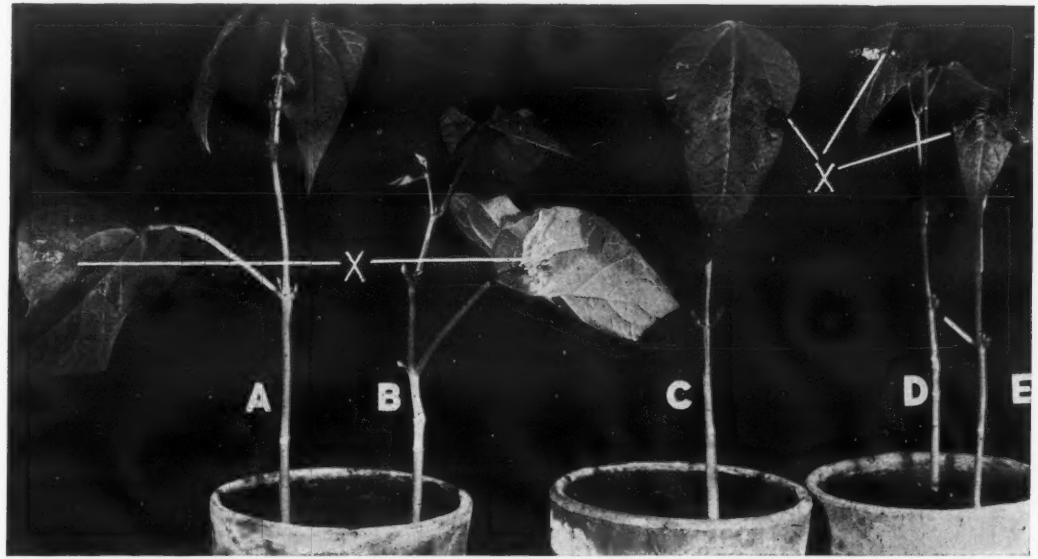


FIG. 17.—Leaves banded with paste on one side at *x*. *A*, 3% paste upperside. Note upward folding. *B*, 1: 10^3 paste on lower side. *C*, third node leaflet, 2.5: 10^4 paste applied to lower side. Note constriction at site of treatment, puckering, savoying, mottling, and downward bending at site of treatment. *D*, *E*, 1.2: 10^4 paste applied to lower and upper sides respectively. Effects as in *C*.

merely a constriction at the site of treatment. This occurred in concentrations $6:10^5$ and greater. The surfaces of such leaves were savoyed and puckered, and all imaginable types of rolling and curling, as well as under-development, were noted comparable to those characteristic of the pseudogalls incited by leaf insects. The color below the strips changed, ranging from a mosaic of different shades of green to mosaics of green and yellow (fig. 17*C–E*). The same results were obtained on young leaves of the second node. In many young leaves the treatment caused negative bending of the midrib resulting in upward or downward flection (fig. 17*C–E*) of the distal half of the leaf. The affected part of the midrib developed tumors with roots, especially at concentration $1.2:10^4$.

Young and aging leaves treated with 3 per cent paste yellowed and dried prematurely. This and premature death of decapitated and treated hypocotyls are the only instances of gross lethal effects from treatment with chemically pure heteroauxin.

Studies of the effects of higher concentrations on various parts of the bean and of various concentrations on the germinating seed are under way.

Tomato

In preliminary experiments with 2 and 3 per cent paste, young tomato plants showed whitening, diameter increase, and prompt adventitious root development proportional to the concentration. Decapitated top internodes showed similar responses. Further experiments were made on tomato seedlings and older plants. The tomato is not so responsive either in rate or amount to heteroauxin as is the bean. Dilutions above $1.5:10^5$ produced no gross responses in aerial parts, whereas the bean reacted to dilutions to $4:10^6$. No experiments were made on tomato roots.

Hypocotyls treated with pastes applied unilaterally in dilutions up to $1.5:10^5$ bent negatively, swelled locally, but gave little diameter increase. Whitening was less marked than in the bean. Root development, however, was more active. Decapitated hypocotyls swelled locally, increased in diameter, and developed surface callus.

Epicotyls treated in the first, second, third, and fourth internodes, as well as petioles, reacted as did the hypocotyl to unilateral ap-

plication or following decapitation. Band applications produced the same effects but no bending.

Application of pastes to the stem produced not only tumors and roots, but epinastic growth of the leaves as well (fig. 18*B*). In the latter respect the tomato is more reactive than the bean.

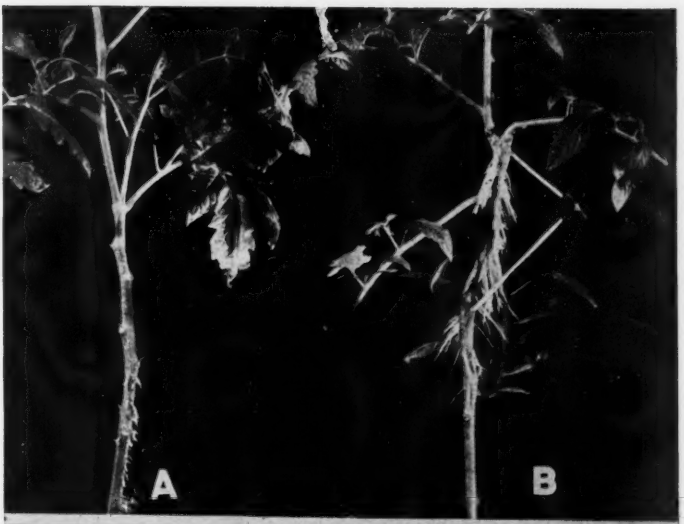
Tomato seedlings grown in sand on a nitrogen-free nutrient solution had ceased growth at time of application with 3 per cent paste. Whitening and later local swelling set in, and growth continued for a considerable period, the tumor acting as a new growth region.

Treatment with agar culture blocks

Blocks of three months old dextrose-tryptophane and dextrose-tryptophane and peptone and two days old dextrose potato agars on which *P. tumefaciens* had been cultured were used in these experiments. Although thoroughly rinsed, no attempt was made at sterilization. Each block was fastened to the surface of decapitated bean hypocotyls by means of fine glass capillaries run through the agar into the hypocotyl. All agars on which *P. tumefaciens* had grown produced swelling at the tip of the hypocotyl. In some instances the first effect was constriction at the very top. The most pronounced growth effects were produced by the dextrose tryptophane agar and the least by the potato dextrose agar. (Possibly this was due to the youth of the culture.) Uninoculated control agar blocks were negative. Cultures of *P. tumefaciens* applied to the surfaces of decapitated hypocotyls caused similar results but less rapidly. Obviously *P. tumefaciens* either liberates a growth substance into its culture medium, or by liberating enzymes changes the ingredients of the medium into growth substances.

Extracts of P. tumefaciens

Aged cultures of *P. tumefaciens* grown on potato dextrose agar in Kolle flasks, then treated with merthiolate or ether extracted, gave faint if any reaction when applied to young tomatoes. All extracts from broth culture were active but varied greatly in strength, as would be expected in such crude preparations. Three weeks old cultures were less potent than those three months old. This activity has been retained during the six months period since their prepara-



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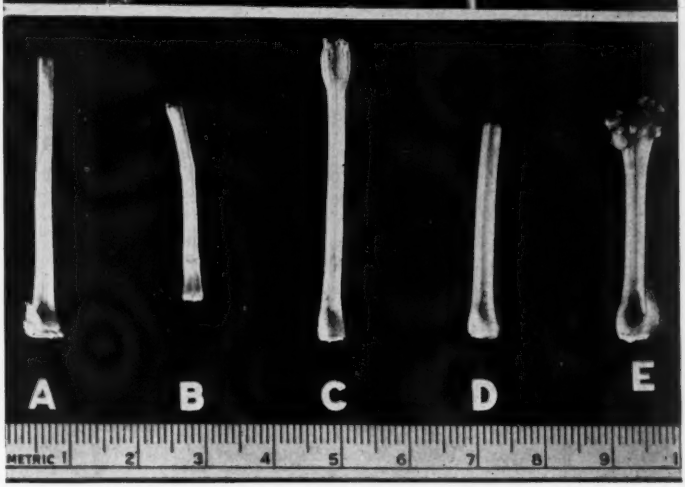

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FIGS. 18, 19.—Fig. 18, *A*, control plant grown in dry air and then placed in Wardian case with high humidity; *B*, plant treated identically but inoculated at *x* with 1: 10^3 heteroauxin paste. Note tumor, epinasty, roots, retention of leaves. Identical effects obtained with young tomato plants treated with extract of *P. tumefaciens*. Control extract produced no effects. Fig. 19, decapitated second internodes of bean. *A*, control; *B*, treated with extract of non-inoculated dextrose-tryptophane medium; *C*, treated with extract of *P. tumefaciens* grown in dextrose-tryptophane medium. Note swelling, diminished near surface. *D*, treated with water paste; *E*, treated with 1: 10^3 heteroauxin paste. All kept in moderately moist air for 3 weeks.

tion. The most active, and consequently used for most experiments, was the extract of the three months old culture in the medium without peptone. It caused pronounced curvature of *Avena* coleoptiles and gave a marked pink in the specific test for heteroauxin. On tomato seedlings it caused local killing, extensive diameter increase, local whitening, and positive bending when applied to the first internode. Roots appeared here as swellings below the surface and at midribs and lateral veins of inoculated leaves. Stem applications caused similar responses in larger tomatoes. When applied to decapitated apical internodes, epinastic bending of leaves ensued.

Application to the second internode of the bean resulted in whitening, diameter increase, root production, and usually positive bending (fig. 14*C*, taken after internode had straightened). Negative bending occurred only in two cases. In the young hypocotyl similar effects ensued, although the bending was always negative (fig. 20*C*). Elongation was much retarded as in the application of 3 per cent heteroauxin paste. Treatment of decapitated hypocotyls and internodes led to responses not noted after application of heteroauxin—a real or apparent constriction at the surface of the stump followed by swelling below and later by less rapid and extensive diameter increase in the constricted zone (fig. 19*C*). The end results resembled those obtained by CZAJA (7) in decapitated *Helianthus* hypocotyls treated with heteroauxin and water paste in different concentrations and combinations. The ether extract of the noninoculated medium produced no effects when applied to plants (figs. 14*B*, 19*B*, 20*B*).

These experiments are less comprehensive than those with heteroauxin. The results are not directly transferable because the extracts may contain active substances other than heteroauxin. Application of some of the extracts to apical tissues of the tomato resulted in killing, and on young internodes of the bean in temporary positive bending, indicating either that the concentration of the heteroauxin is greater than 3 per cent or that other more effective substances are present. Sections of internodes with positive bending reveal no killing of tissues, indicating that the young bean internodes are less sensitive than those of the tomato to killing by some ingredient of the extracts. The wounding experiments with acetic acid indicate

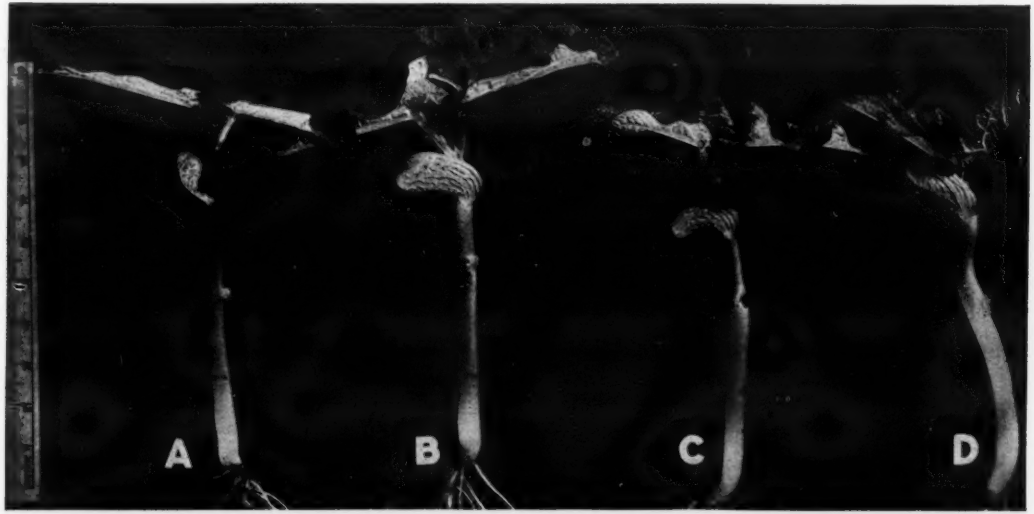

FIG. 20.—*A*, control hypocotyl treated unilaterally with water paste. *B*, control treated unilaterally with extract of non-inoculated dextrose-tryptophane medium. *C*, treated with extract of dextrose-tryptophane culture of *P. tumefaciens*. Black material is the extract. Note local depression due not to killing but to locally lessened swelling, peripheral swelling and whitening, general diameter increase, retarded elongation of hypocotyl, and retarded expansion of epicotyl. Photographed after 12 hours when initial bending had been almost rectified. Sections of figs. 21 and 22 cut from such a site after 8 days. *D*, unilateral application of 3% paste after 10 hours. Note bending being rectified, diameter increase, local swelling, whitening, retardation of epicotyl development. Black line indicates soil line.

that the primary growth responses incited by the extracts are initial growth responses to these substances and not secondary growth responses incited by local necrotization.

In initial experiments the extracts were over-diluted in lanolin, as indicated by feeble responses of the plants. From later experiments and written information from Dr. F. E. GARDNER, it is further concluded that these poor results were also due to age of test plants and unfavorable growing conditions. In later experiments mixture of the extracts with lanolin before application might have extended their period of activity.

TREATMENT WITH GALL EXTRACTS

Chloroform extracts of *P. tumefaciens* incited tomato galls, and control extracts of the stem and leaves when applied to the bean caused local killing. The gall extract when applied with puncture caused slight local swelling and whitening on the hypocotyl. On the second internode it produced slight swelling and slight positive bending; stem and leaf extracts were negative. On tomato seedlings killing and doubtful negative bending occurred. On older tomatoes killing was the only noted response. These tests were all essentially negative. They are being repeated.

HISTIC STUDIES

Samples of tumors induced by bacterial extracts and by 3 per cent heteroauxin paste were collected at frequent intervals and placed in Navashin's solution preliminary to histic studies. This paper will deal only with eight days old material produced by treatment with the extract. Treatment with 3 per cent heteroauxin paste when applied in dosage of 0.005 cc. produces essentially the same effect. The sections reproduced in figures 21*C* and 22*B*, *D* are from tumors such as shown in figures 7*A* and 20*C*. The hypocotyl was inoculated just below the arch before straightening. In the mature hypocotyl the lesion finally was 2–3 cm. above the soil level. The gross symptoms are those discussed in the section on treatment of the hypocotyl.

Initial bending of the hypocotyl is due to cell enlargement, most pronounced on the treated side and along the radial and tangential axes of the cells; cortex, including the endodermis, and the rays and

pith are most affected. While the effect was most marked in the cortex, under and adjoining the site of application, the entire hypocotyl was affected. This was evident in change opposite the site of application (fig. 22*A*, *B*). Clearing is due to disappearance of chlorophyll, but even more to increase in volume of the intercellular spaces of the cortex (figs. 21*C*; 22*B*, *D*). This increase was brought about by such marked enlargement of cells of the cortical parenchyma that some adjoining cells began to pull apart at their middle lamellae (fig. 21*C*). The resultant tissues are identical with the chemically induced pathic tissues which KÜSTER (**17**) classified as hyperhydric. In the latter stages of intumescence complete loss of continuity between cells of the cortical parenchyma ensued. Meanwhile cortical cells adjoining the endodermis and the endodermal cells enlarged greatly radially and less tangentially, and began to divide. Division was mostly tangential, but also radial and less frequently oblique, the new walls being laid down within the greatly stretched old walls (fig. 22*A–D*). Other cortical cells divided and the resultant tissue resembled an internal callus. The nuclei of the affected cells were greatly enlarged.

The cells of the cambium, primary phloem, and rays are likewise characterized first by dimensional, later by numerical increase. Eventually the phloem gave rise to new meristems and bundles as described for decapitated and heteroauxin treated second internodes of *Phaseolus vulgaris* (**16**). The pericycle appeared relatively inactive. Cells bordering the cavity, including the endodermal cells or their tabular derivatives, at times developed as long, hairlike cells into the cavity (fig. 21*C*). These cells simulated the much elongated cells characteristic of many foliar intumescences (**17**) and of surface callus. Other cells bordering or distant from the cavity at times divided in various planes. Continuity of the epidermis was rarely broken, apparently because of continued radial divisions (fig. 22*B*, *D*). Cortical cell tissue, including the endodermis, on occasion produced the isolated or grouped, short, thick walled elements which KÜSTER (**17**) designated wound tracheids. The endodermis and adjoining cortical cells at times gave rise to islands of meristems with resultant extrafascicular vascular bundles as described for incised internodes of *P. multiflorus* by SCHILBERSKY (**29**) and for de-

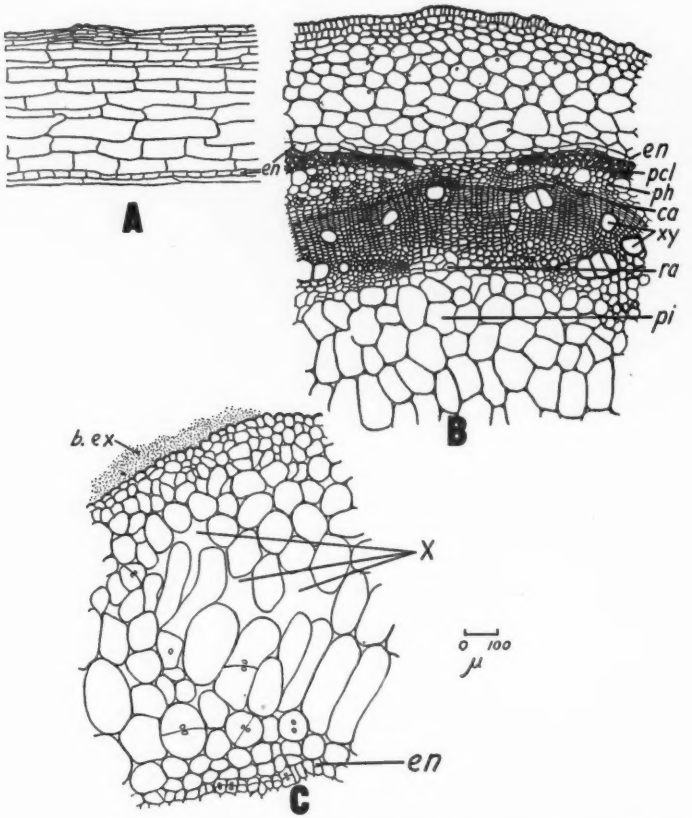

FIG. 21.—*A*, longitudinal section of cortex of normal hypocotyl of bean; *B*, transverse section at same level as figs. 21*C* and 22*B*, *D*. All same age. Secondary thickening and development of thick walled elements in *B*. *C*, transverse section through region of hypocotyl treated with bacterial extract (*b.ex*) fixed 8 days after treatment. Cell division in epidermis and subepidermal layers, more pronounced under paste; less thickening of axis immediately under paste; enlargement of most cortical cells, division in endodermis (*en*) and cortical layer adjoining it; large cavity at *x* being filled with hair-like callus cells from bordering cells. Cavity due to excessive enlargement of cortical cells with resultant tearing of middle lamellae and plasmodesmata. Other evidences of internal callus.

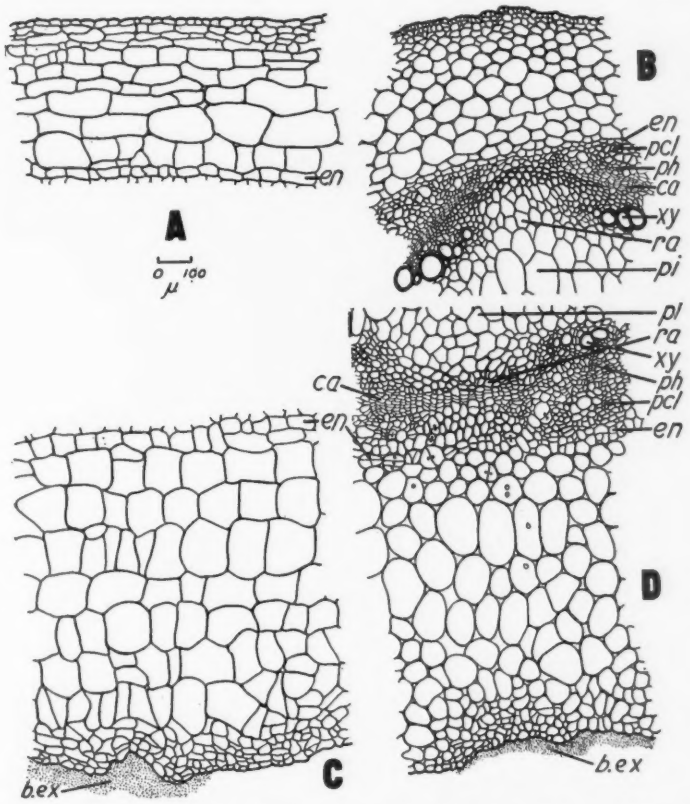

FIG. 22.—Sections through hypocotyl treated unilaterally with bacterial extract (*b.ex*). Same age as other sections of figs. 21 and 22, 8 days after treatment. *C*, *D*, longitudinal and transverse sections of treated side; *A*, *B*, exactly opposite, not treated. Note general diameter increase in *D*, enlargement of cortical cells, especially in radial and tangential planes; increase in intercellular space in cortex; less enlargement but more division immediately below *b.ex;* cell division in endodermis (*en*), pericycle (*pcl*), cambium (*ca*), phloem (*ph*), ray (*ra*), and pith (*pi*); less differentiation and maturation in *B* and *D* than in *B* of fig. 21; change in orientation of cell axis comparing fig. 21*A* with 22*A*, *C*, and fig. 21*B*, *C* with 22*B*, *D*.

capitated and heteroauxin treated second internodes of the Red Kidney bean by KRAUS, BROWN, and HAMNER (16). When lateral roots developed in or near the treated region, cortical cells abutting the endodermis, the endodermis, pericycle, and rays seemed to be involved in their initiation as reported for secondary roots of the Leguminosae and Cucurbitaceae by JANCZEWSKI (10).

The small cells of the epidermis and the adjoining cells evident in figure 22*A–D* indicate that cell division has taken place in the outer cortex. Whether these and other cell divisions noted in treated hypocotyls are direct responses to the applied heteroauxin or are secondary reactions to initial cell enlargement and other effects induced by the bacterial extracts and by heteroauxin has not yet been determined. There are many cells, especially in the endodermis and adjoining cortical layer, which enlarge and then go through a series of divisions without continued enlargement. Further study of the material fixed hourly immediately after treatment may reveal that cell division is an initial response to certain concentrations of these substances. WEHNELT (33) and JOST (11) assert that it is.

The affected tissues are less differentiated than the normal. In the specimen represented in figure 22*A–D* differentiation is less advanced than in the untreated specimen shown in figure 21*A*, *B*. The endodermis has dedifferentiated; and differentiation of the stele is below normal. Phloem fibers have failed to mature, as have many of the prospective woody constituents of the xylem.

The sections of figures 21*C* and 22*D* reveal that the effects were most pronounced below the site of application. This probably is due to greater concentration of the substance (*b.ex*) in that region. It probably indicates that other effects may be anticipated from different dosages and concentrations. Immediately below the site of application of 3 per cent paste and of the bacterial extracts, there was less swelling than at the periphery of the application. There also was more evidence of division in the outer cortex (figs. 21*C*, *b.ex.*; 22*C*, *D*). This indicates that high concentrations tend to induce less cell enlargement (or none) and more (or only) cell division. Since high concentrations seem to induce more cell divisions than low ones, the high incidence of divisions in the stele, endodermis, and adjoining row of cortical cells may indicate an increased concentration in this

region, possibly owing to meeting of the autoauxones and the heteroauxones at the endodermal barrier.

Discussion

The results indicate that in the Red Kidney bean the cotyledons and young epicotyl are initial sources of growth hormones; further, that all aerial vegetative parts if green are able either to generate growth substances or to convert their precursors into hormones. This inference is based on the observed increase in length of decapitated hypocotyls in the light and of plants both decotyledonized and deepicotyledonized. Transport of growth substances proceeds mainly toward the central axis from the peripheries of the aerial divergencies. In the central axis transport is mainly basipetal but also acropetal and transverse. While decapitated hypocotyls and first internodes elongate, decapitated second and third internodes do not. Possibly those here employed were too old, even though some were only 1 cm. long when decapitated. Most were 1.5 cm. long at that time.

The inward and downward flow of hormones or hormone precursors from divergences into the axis is somehow associated with inhibition of development of axillary buds of the cotyledonary and higher nodes. This effect is demonstrated by development of these buds after spontaneous blasting of the leaves of the embryonic plumule or after decapitation of the internodes. Inhibitory effects of the intact leaf are evident in increased elongation of first internodes after petiole decapitation.

The observation that leaves fold upward whether treated on the upper or lower side needs further investigation, also the observation that application of heteroauxin to the upper side of a leaf leads to lengthening of the petiole while application to the lower side leads to shortening. Possibly position of xylem and phloem in the veins and petioles is in some way associated with the phenomenon.

The bean is highly disposed following wounding to formation of surface and internal callus and their derivatives; to adventitious root development and root fasciation; and to root regeneration. It is not disposed to formation of adventitious shoots or to non-nodal shoot regeneration. Moderately old hypocotyls grown above soil

and later encased in sand will develop roots in four rows throughout their full length if the sand is kept moist. Internodes failed to respond to this treatment with root development.

No detailed discussion will be attempted of implications of these results relative to polarity and correlation in the bean; that will be done in a later paper. The present report is concerned mainly with the etiological implications of the experiments and data. These exemplify basic principles discussed earlier (**21, 22**). In the second paper, hormones acting as external agents were recognized as pathogenic factors. The experiments and data of the present paper show clearly that disturbances in plants following application of a foreign chemical agent, heteroauxin, with or without wounding, are due to a causal or etic complex, which comprises (*a*) the foreign agent, and (*b*) the disposition of various cells, tissues, tissue systems, and organs of the plant to react at a particular moment to a particular concentration and amount of the agent. Four factors that condition the disposition of a cell, tissue, tissue system, or organ to react may be recognized: (1) hereditary constitution, genic and non-genic; (2) past experience; (3) correlative factors (effect of one part on another part); (4) environal factors (factors outside the whole affecting it at the moment under consideration). The relation of these factors to one another is schematically treated in an earlier paper (**21**).

Individuals of different species, bean or tomato, reacting differently to the same concentration or amount of heteroauxin, indicate the role of hereditary factors, genic and non-genic.

Differential reaction of the same organ, tissue, or cell of the bean at different stages of its development or following different culture conditions (light, humidity, temperature) prior to treatment with a given concentration of heteroauxin indicates the role of the past history factor.

The role of correlative factors is indicated by differential susceptibility of the same organ, tissue, or cell of the bean to a given concentration of heteroauxin in the presence or absence of other members of the whole.

Differential reaction of the same organ, tissue, or cell of the bean to the same concentration of heteroauxin under different environ-

al conditions at and subsequent to the moment of application indicates the role of environal factors.

Given (*a*) disposition to react to a particular inciting agent, in this case an external one, in the form of a particular concentration of heteroauxin, and (*b*) the application of this concentration, then (*c*) a characteristic reaction, cell enlargement with or without division, or possibly cell division alone, sets in. Next this event, playing the role of an internal factor, may become the inciting factor which releases other reactions according to the dispositions of the cells involved. This causal complex gives certain tissues and cells opportunities to realize qualitative and quantitative potentialities of cell enlargement, division, and differentiation which are not stimulated to or are inhibited from expression in the course of normal development.

Usually the inciting agent or factor, especially if it is an external one, is called the cause (**22**). Brevity is the only justification of this usage. It ignores the fact that each constituent of the etic complex is a necessary but not a sufficient cause of the event. Each in turn will appear as the cause if added or withheld while the others are kept constant.

These considerations emphasize the role of living protoplasm in reactions induced by heteroauxin and other growth substances, a consideration repeatedly stressed by JOST (**11–13**). However, they do not commit one in the stimulus dispute carried on by FITTING,[4] WENT (**34**), and JOST.

Since wounding or application of bacterial extracts which contain heteroauxin (and possibly other growth substances) and application of pure β-indoleacetic acid lead to similar effects in the bean and tomato, the hypothesis is suggested that disturbance of the usual growth hormone concentration of the affected plant is one constituent of the causal complex of the cell enlargement and division characterizing these effects. Their induction by application of heteroauxin and the apparent partial substitutability of heteroauxin for the auxines of the cotyledons and epicotyl suggest that the local growth substance concentration in the region of the prospec-

[4] FITTING, H., Die Hormone als physiologische Reizstoffe. Biol. Zbl. **56**:69–86. 1936.

tive tumor rises above the norm, but that this hyperauxony is not sufficient either to inhibit cell enlargement or division or to induce necrosis. This part of the hypothesis could readily be tested by comparing the auxone contents[5] of tumors, galls, and normal tissues.

Disposition of the affected organ, tissue, cell, or cell part to respond with enlargement, division, or differentiation to a particular hyperauxony is the other constituent of the causal complex of growth reaction to wounding, bacterial extracts, and heteroauxin applications.

In gall formation the same etic complex is operative but in addition a similar causal complex for the infecting agent must be considered (**21, 22**). If the organism consists of only one cell, intercellular correlative factors do not enter the complex so far as the parasite is concerned. In gall formation, the hyperauxonism probably is not a brief but an extended condition due to continued presence of the parasite and the disturbances which it induces. The parasite probably not only furnishes a more or less continuous supply of heteroauxones, but through abnormal growth or lethal effects of the host disturbs normal production, activation, and transport of autoauxones. In starting new and abnormal growing centers, the parasite probably creates additional sites of increased autoauxone

[5] The field of growth substances is in need of a tentative classification and nomenclature. Since the terms auxin and heteroauxin have been applied to specific chemical agents, and since diverse chemical agents act as growth substances, the collective term auxins is not satisfactory. Proceeding from the Greek verb auxein, which means any increase, amplification or waxing, we have used auxone as a generalized term for all growth substances, including the reproductive hormones that affect growth. It includes the A and B growth substances. In case specific auxones should be found that induce dimensional increase (cytoauxesis), numerical increase (cytomeresis), complexity increase (cytoplasesis), or plasma increase (cytoplasmesis) of cells, they can be designated cytoauxesones, etc. Any auxone inducing more than one of these reactions may be said to be cytoauxegenic, cytomeregenic, etc. Auxones influencing reproduction may be designated genesones. Excess or deficiency in any of these processes or substances may be indicated by the prefixes hyper or hypo respectively.

Some auxones are produced by the individual whose development they affect; these are autoauxones (auxin a and b). Others apparently do not affect growth of the plant producing them but of other plants; these are heteroauxones (heteroauxin). Heteroauxones also include growth substances not known to occur in plants (indolepropionic acid, indolebutyric acid, etc.).

No confusion will arise if the ending 'one' is avoided in coining specific names of growth regulating substances.

production. Internal wounds resulting from death of some gall cells may induce local hyperauxonity, with resultant cell enlargement and division, as in simple wounding. Since *P. tumefaciens* usually becomes initially effective only through wounds, its presence may in part enhance and extend spatially and temporally a hyperauto-auxony instigated by the initial wound.

In the highly differentiated galls incited by some insects, the kind of auxones produced by, in, or on the parasite as well as the mode, duration, and quantity of their production probably are more significant than in the relatively simple callus-like galls (**18**) induced by *P. tumefaciens*. It is possible that the normal bacterial flora of the exterior and of the intestinal tract and mouth parts of insects play roles in the production of heteroauxones on or within the insect's body, or that the insect itself produces auxones in the course of its metabolism. If heteroauxones are produced from ingested food material within the insect's intestinal tract, the frass of insects should contain heteroauxones. LARUE'S report (**19**) that feces of insects produce tumors suggests that this hypothesis may be a step in the right direction.

Since heteroauxones produced by non-gall forming organisms incite tumor formation in plants, not parasitized by these organisms, and since a synthetic heteroauxone or wounding may lead to tumor production, it appears that gall production in a particular host by a particular organism is initially conditioned by factors determining specificity of parasitism or infection rather than by specificity of a chemical mechanism involved in growth disturbances.

The event of infection must be subjected to the analysis here outlined. Its causal analysis involves consideration of two interacting causal complexes, the host, organ, tissue, cell, or cell part being infected and the individual, organ, tissue, or cell doing the infecting. For the first, the disposition to be or not to be infected by a particular species, individual, organ, tissue, or cell must be analyzed in terms of the four causal constituents just discussed. For the infecting agent, its disposition to infect or not to infect the particular individual or part under consideration must be resolved in terms of these four factors (**21, 22**).

Obviously capacity to infect and capacity to incite growth re-

sponses or other pathic effects are not due to identical causal complexes, nor are capacity to be infected and capacity to respond to infection with one or more of the various manifestations of injury or injury reaction.

Many observations indicate that infectivity must be differentiated from more severe types of pathogenicity or virulence (**22**). The least severe obvious injury resulting from infectivity is loss of purity or contamination of the host and of the parasite. COOK (**6**) has shown that tomato plants may be heavily infected with *Fusarium lycopersici* without displaying other obvious evidences of injury. If this fungus does not produce its characteristic toxic substances, the symptoms characteristic of tomato blight do not develop. KANE and LINK (**14**) showed that while *F. lycopersici* is host specific as to infectivity, its toxic substances are not in that they induce typical blight and wilt injury in plants never parasitized by the fungus.

The observation that fungi and bacteria that incite galls produce heteroauxin suggests the hypothesis that heteroauxin or other heteroauxones play important roles in the production of galls by parasites. Studies are under way to determine whether *Erwinia amylovora*, *Phytomonas rhizogenes*, *Rhizobium phaseoli*, *Taphrina deformans*, other species of *Taphrina*, and *Ustilago zeae* produce heteroauxin or other heteroauxones in culture.[6] Possibly mycorrhizal fungi serve their host by the formation of heteroauxones.

Studies also are under way to determine whether galls such as the clubs induced by *Plasmodiophora brassicae*, the nodules of the bean, leaves and fruits infected with *Taphrina* spp., rust, smut, and virus

[6] Note while reading proof:

Using the procedure and apparatus described by GALLAGHER, KOCH, and DORFMAN (GALLAGHER, T. F., KOCH, F. C., and DORFMAN, R. I., Procedure for quantitative extraction of sex hormones from urine. Proc. Soc. Exp. Biol. and Med. **33**:440–444. 1935), ether extracts were prepared from these organisms. Preliminary tests gave the color reaction (ferric chloride, hydrochloric acid, amyl alcohol) characteristic of β-indoleacetic acid for these organisms in the following ascending order: (1) *E. amylovora*, (2) *P. tumefaciens*, (3) *Ustilago zeae*, (4) *Rh. phaseoli* (strain 400 Wis.), (5) *Taphrina deformans*, (6) *P. rhizogenes*, (7) *Rh. phaseoli* (strain 407 Wis.), (8) *Rh. phaseoli* (strain 403 Wis.). We have not had time to make the coleoptile test. We are indebted to H. R. ROSEN for culture of no. 1; E. C. STAKMAN for culture of no. 3; E. B. FRED for cultures of nos. 4, 7, 8; A. J. MIX for culture of no. 5; and to A. J. RIKER for culture of no. 6. We are indebted also to PROFESSOR KOCH for use of his laboratory and equipment and for advice and help.

infected organs, and several insect galls have a higher auxone content than the healthy organs.

The observation that high concentrations of auxones bring on necrobiosis and necrosis suggests that fungi and bacteria may necrotize by contributing to lethal hyperauxony, through adding their heteroauxones to the host's autoauxones, stimulating host production of autoauxones, or blocking autoauxone transport and causing local accumulation.

That heteroauxin applied to a plant can either substitute in part for its autoauxones or augment their action, and the well known fact that soil fungi and bacteria produce heteroauxin, suggest that some of the beneficial effects of humus soil may be due to the auxones of decaying plant debris or soil flora.

Auxones are harmful to roots in relatively high dilutions, and roots of the bean treated with certain concentrations of heteroauxin resemble those of certain bog and muck soil plants. This suggests that hyperauxony of these substrates may possibly be in part responsible for the characteristic roots of some of the plants grown in them.

Given a foreign organism able to infect a particular host and capacity of this parasite to form heteroauxin, the presence of the parasite need not lead to gall production if the metabolic status of the infected tissue is such that relatively little tryptophane or tryptophane containing proteins is present. The metabolic status and nutrition of both the parasite and host and everything that influences them undoubtedly are factors in the etic complexes of galls.

It has long been surmised that gall incitants may be effective by means of chemical agents. MALPIGHI in 1672 (**26**) devoted a portion of his anatomy of plants to galls. He attempted an explanation along physiological and chemical lines. He postulated that juices introduced by the insect act as foreign chemical agents which lead to a new fermentation (in modern terms chemical change) or to a new movement (in modern terms electronic vibrations, etc.) in the injured region. Nutritive sap accumulates abnormally in this region. The local disturbance in turn incites fermentation in this accumulated sap leading to the dimensional and numerical increase in parenchymatous cells which he had observed in the galls. Here we

have a physiological chemical theory by the first of the pathological plant anatomists and phytoparasitologists. MALPIGHI also concerned himself with legume nodules but was unable to discover any insect within them, although he reported a peculiar content.

The mechanism whereby *P. tumefaciens* induces galls destructive in economic plants has stimulated much speculation and much research. SMITH was engrossed in these endeavors and did much to indicate that the organism might be effective through chemical agents. His last book (**31**) contains an excellent summary and full bibliography. BECHHOLD and SMITH (**2**) reported finding a "tumefaciens plastin." NĚMEC (**27**) reported that bacteria produce callus forming substances. This phase of the crowngall problem is reviewed by RIKER and BERGE (**28**) and needs no further review here.

HABERLANDT'S findings (**8**) of wound hormones and recent hormone work indicate definitely that hyperauxony may be a factor in tumor and gall production. Demonstration that many fungi and bacteria produce β-indoleacetic acid in the course of their metabolism is highly suggestive that this and similar substances may be some of the chemical agents effective in gall formation.

In our work so far, the Salkowski color test, which is considered specific for β-indoleacetic acid (**1, 15**), and the comparable effect on plants of heteroauxin and the bacterial extracts are the basis for assertion that *P. tumefaciens* produces heteroauxin. Chemical identification by purification and analysis are essential for final proof. Further work is necessary to determine whether heteroauxin is the only auxone formed by *P. tumefaciens*. Possibly the better extraction technique now employed will enable us to obtain greater yields. They must be larger to permit analysis, because KÖGL and KOSTERMANS (**15**) started with 50 kg. of yeast and finished with only 9 mg. of β-indoleacetic acid.

Our gross observations on the bean confirm JOST and REISZ'S (**12**) more refined findings for *Avena* coleoptiles, that both concentration and amount of auxone are factors in the response; that heteroauxone enters the plant within the first two hours, and that it moves upward, downward, and transversely; that heteroauxones may substitute for or augment a plant auxone; and that temperature and moisture are conditioning factors. They also show that the heteroauxin effect is greatest in the dark.

Heteroauxin apparently does not pass heavily cuticularized or suberized walls. Possibly this accounts for the indicated accumulation of auxones in the endodermal region of plants to which heteroauxones are applied.

The histic studies of the hypocotyls indicate that the lower concentrations of auxones induce auxesis, while higher concentrations induce auxesis and meresis. The higher concentrations also are able to modify plasesis leading to dedifferentiation and redifferentiation (metaplasesis) of the endodermis, probably by influencing cell enlargement and division. Through these effects they also lead to hypoplasesis of the stele, evident in less development of secondary xylem and phloem and diminished differentiation and maturation of primary xylem and phloem constituents. Secondary hyperplasesis is evident in the extrafascicular bundles observed by us for the hypocotyl and reported for the internodes by KRAUS and associates (**9, 16**).

Heteroauxin not only induces functional injuries but indirectly through growth disturbances leads to material injury in the form of tearing of middle lamellae and plasmodesmata in the formation of cortical cavities. The mechanism of necrosis of leaf tissues has not yet been investigated. Whitening and yellowing indicate that chlorophyll is either destroyed or its synthesis stopped by certain concentrations of heteroauxin. The effects of this one substance in different concentrations and amounts on the various organs of the bean illustrate practically all the major types of functional and material injuries[7] known to the phytopathologist.

Are not the results of these experiments further indications that circumscription of phytopathology on the basis of the germ theory of disease has outlived its usefulness and that mycologic concepts and techniques alone are not adequate for its development? (**21, 22**).

Summary

1. Differential behaviors of axes of bean, tomato, and *Bryophyllum* inoculated with *Phytomonas tumefaciens* are due probably to differences in growth patterns of the axes of these plants.

[7] Injury designates destruction or other impairment of functional capacity (BOT. GAZ. **97**:686–688, 1936).

2. Much of the overgrowth may be due to responses to wounds essential to the course and completion of the infection process.

3. The bean is highly disposed following wounding to formation of surface and internal callus and their derivatives, adventitious roots, and root regeneration. The roots are disposed to fasciation. The bean is not disposed to adventitious shoot formation or to non-nodal shoot regeneration. Moderately old hypocotyls developed in air and then encased in wet sand will develop adventitious roots through the full length of the organ.

4. Occurrence of these events at the wound and above it indicates that growth substances play a role in their enactment.

5. Growth substances apparently are formed or activated in all aerial vegetative members of the bean and transported to the central axis where they move mainly downward, but upward and transversely as well.

6. Elongation of hypocotyl, internodes, and development of axillary buds are retarded or inhibited by hormones from divergences of segments of the axis above them.

7. Disposition of an organ to enact these events is conditioned by its hereditary constitution, age, presence or absence of other organs, metabolic status, and environal factors (temperature, moisture, and light).

8. Enactment of these events is influenced by experimentally introduced growth substances (heteroauxones). These seem to be able to augment or to substitute in part for the native growth hormones (autoauxones) of the bean.

9. Different concentrations and amounts of heteroauxin applied to different organs of the bean and tomato under different conditions are able to elicit almost the entire gamut of injuries and injury reactions known to the phytopathologist. This includes tumors.

10. *Phytomonas tumefaciens* produces β-indoleacetic acid (heteroauxin) when grown in dextrose-tryptophane or in dextrose-tryptophane peptone liquid or solid (agar) medium. The crude ether extract of the non-inoculated media does not contain heteroauxin. The chemical Salkowski and the biological *Avena* coleoptile tests were used.

11. The crude ether extract elicits symptoms from bean and tomato similar to those induced by inoculation with *P. tumefaciens* or by treatment with heteroauxin.

12. Heteroauxin is a chemical agent by means of which, possibly in conjunction with others, *P. tumefaciens* incites galls.

13. Unequal amounts of 3 per cent heteroauxin paste and of crude extract of *P. tumefaciens* applied to the hypocotyl of the bean elicit practically identical histic and cytic effects. These are cell enlargement, cell division (possibly a consequence of enlargement), and as a consequence of these suppression of normal differentiation and maturation but genesis of new meristems with new differentiations in abnormal sites. Schizogenous cavities are formed, followed by filling with peripheral callus. Differential response by the same tissue or cell to different amounts and concentrations and different responses of different tissues or cells to the same concentration or amount are indicated. Most of the abnormal tissues and cells are pathic in the sense that they have less adaptive capacity for unfavorable environal conditions than have the normal healthy ones.

14. This study indicates that biology must use the concept "causal complex" in attempting analysis of problems in normal and abnormal, healthy and pathic morpho-, organo-, histo-, and cytogenesis. Each constituent of the causal complex is a necessary but not a sufficient cause for the event under consideration.

15. A tentative nomenclature and classification of growth substances are proposed.

16. Hypotheses are advanced suggesting partial explanation of legume nodules, insect galls, forms of roots of bog, muck and mycorrhizal plants, as well as beneficial effects of humus soils, in terms of auxones.

17. Relation of infectivity and other expressions of pathogenicity are discussed.

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CURRENT LITERATURE

Methods in Plant Physiology, a Laboratory Manual and Research Handbook. By W. E. Loomis and C. A. Shull. New York: McGraw Hill, 1937. Pp. xviii+472. Figs. 92. $4.50.

As stated in the preface, this book is an attempt to bring together material which will be useful to the teacher and student in plant physiology, as well as to the research worker in this and allied fields. To a large extent these purposes are fulfilled.

The first 227 pages are devoted to a description of 182 standard experiments suitable for a laboratory course in plant physiology, including experiments relative to water relations of plants, plant nutrients, diffusion, colloidal phenomena, photosynthesis, pigments, foods, respiration, enzymes, growth, and movement. Following the introduction to each chapter, a limited number of pertinent references are cited. A few references relative to certain experiments follow the description directly. Each experiment is followed by one to four thought-provoking questions.

Part II of the text contains directions for biochemical, biophysical, and biometrical determinations which are incident to research in plant physiology. These descriptions give rather detailed information in regard to equipment, cleaning of glassware, filtration, titration, standardization of solutions, chemical analysis of plant materials, freezing point and H ion determinations, and control of light, temperature, and moisture. The last chapter contains thirty-four pages of description and discussion of statistical methods by G. W. Snedecor. A copious appendix of forty pages completes the text.

The language used in the directions and explanations is clear and concise, a commendable factor too frequently lacking in such manuals and textbooks.

In the advanced portion of the text the general procedure which is essential to the problem in hand is carefully outlined, while the general details are left to the discretion of the investigator. This is as it should be, for although certain of the details of any line of investigation may change from time to time, the basic procedure remains the same and its exact fulfilment is essential for significant results in research.

The chapter on statistical methods is a valuable addition in supplement to the text. The student of plant physiology should have at least a brief introduction to this subject, since at the present time statistics apparently occupy an important place in the interpretation of research in biology.

The appendix is one of the most useful parts of the book. In it considerable information and data essential to the study of plant physiology are assembled and condensed into tables.

It is impossible to include in any text all the material that every worker in the field would desire, or to express the knowledge therein to the satisfaction of all. In some cases, however, it seems to the reviewer that the experiments are outlined in a more complex fashion than is necessary. This is illustrated in the directions for the preparation of invertase from yeast. For an intermediate experiment this could be done just as efficiently and in a much less elaborate manner than as described in the text.

The directions for the experiments assume that the average laboratory of plant physiology has all the equipment designated. It is perhaps best, however, to give directions calling for ideal equipment, and then leave to the ingenuity of the instructor the task of replacing or substituting when necessary.

The objections that may be pointed out in the text are minor, however, and the book in its entirety is an excellent one. It is a volume that all who are interested in plant physiology or its allied fields will wish to possess.—E. C. MILLER.

Lehrbuch der Pharmakognosie für Hochschulen, fünfte vollständig umgearbeitet und vervollständigte Auflage. By GEORGE KARSTEN and ULRICH WEBER. Jena: Gustav Fischer. 1937. Pp. vii+420. Figs. 574.

This standard work, now in its fifth edition, has been the principal textbook of pharmacy in Germany for more than twenty years. Even a cursory glance through the book, with its numerous illustrations, many of which are colored, shows the advanced condition of the subject in German universities. KARSTEN, now Professor Emeritus of Botany of the University of Halle, made notable morphological contributions before he began to specialize in pharmacy. His work on the life histories of seed plants and of diatoms is classic, and his extensive work on marine plankton has not yet been surpassed. This broad training has been utilized in the textbook, making more definite and reliable the diagnosis of constituents of drugs. WEBER is giving courses in pharmacy in the University of Würzburg.

The general arrangement of drug plants and the drugs obtained from them follows the taxonomic sequence from the algae to the orchids. There is usually a general description of the plant, followed by morphological and histological characters, and often there is added the microscopic structure of the drug. For the angiosperms, which furnish so much of the material for drugs that all other sources form a comparatively small percentage, there is a subdivision into drugs derived from the root or rhizome, from the stem, bark, leaf, flower, fruit, seed, and other parts. Besides these detailed descriptions there are numerous keys for the determinations of various powders, those from root, stem, leaf, flower, fruit, seed, etc.; an arrangement based upon chemistry, a description of the comparatively few drugs obtained from animals, a list of homeopathic drugs, and an arrangement based upon the diseases for which the drugs are used.

The clear presentation, with such a wealth of illustration of plants in the normal condition and as they appear in drugs, together with complete descrip-

tions of solutions, make the book a good introduction to scientific pharmacy.—C. J. CHAMBERLAIN.

Genetical and Taxonomic Investigations in the Genus Oenothera. By R. RUGGLES GATES. London: Phil. Trans. Roy. Soc. Series B. Vol. 226 (no. 536). 1936. Pp. 239–355. Figs. 73.

This is a presentation of the beginning of a survey of the genus *Oenothera*, mainly in eastern Canada and adjacent territory, through a combination of genetic and taxonomic methods. Seventeen new species, fifteen new varieties, and many smaller variations are described as a result of three years of pedigree cultures from about 100 collections of wild seeds from different localities. In addition, seven previously described species have been recognized in new localities.

New information upon the geographic distribution and relationships of the various forms is presented, indicating a south to north movement in several different lines, and also a coastal series of forms. Six species have produced known or probably trisomic mutations, one species a triploid mutation, and one a periclinal chimera, most of these mutations being directly from wild seeds. Some of the species show "early" and "late" development in the cultures, but the condition is not inherited and appears to be an epharmonic response to environmental conditions occurring at an early stage of the young plant's development. And in several species a new category of evanescent characters appears.

Cytological examination has shown that all these forms possess, without exception, a ring of fourteen chromosomes.

The polymorphic character of the genus, even in areas such as Nova Scotia which were heavily glaciated and apparently contain no other endemic species, leads GATES to conclude that evolution has proceeded more rapidly in this genus than in most others. Gene mutations have been active in supplying the materials for specific differentiation and crossing has also played a part in increasing the number of specific types, since owing to catenation the hybrids breed true. Parallel mutations have also occurred frequently in the different species.—J. M. BEAL.

The Nature and Properties of Soils. By T. L. LYON and H. O. BUCKMAN. 3rd ed. New York: Macmillan Co., 1937. Pp. xiii+392. Figs. 45. $3.50.

Investigators and teachers of plant and soil science will welcome this new edition of a standard and important text, the first edition of which under its present authorship appeared in 1922. The treatment in the present edition follows the general plan of the preceding ones. The nature and properties of soils are not merely treated as such but they are also related to the growth of plants. Edaphology is the name applied to this phase of soil science, in contrast to pedology, which treats soil science as a pure science and does not make the application to the plant. The present edition brings the subject up to date. Considerable

new material has been added. One whole chapter is devoted to the newer knowledge of the nature of colloidal clay and of base exchange. Considerably less space is given to soil water than in the second edition.

It is interesting to note the development of edaphology as reflected in the relative emphasis of the three editions on various subjects. For example, base exchange received relatively little attention in the first edition, while a chapter each was devoted to soil aeration and soil temperature. The latter two subjects are given rather slight consideration in the two later editions, and are treated in connection with other subjects. On the other hand, the important and rapidly developing subject of base exchange is given much emphasis, especially in the last edition. While it is essential that the newer developments be given adequate treatment, it seems unfortunate that in so doing the consideration of other important subjects should be so severely curtailed.—S. V. EATON.

Die kontraktile Zelle der Pflanzen. By SILVIA COLLA. Berlin: Gebrüder Borntraeger, 1937. Pp. ix+168. Figs. 77. Rm. 12.

This work is volume X of the Protoplasma Monographien. The introduction develops the historical and current points of view regarding contractility. The first chapter discusses the material and the research methods used in the study of cellular contractility. Other chapters discuss the morphology of contractile cells, the mechanisms of movement of contractile organs, the various phases of contraction, the laws which govern the contraction and movement of isolated cells, the physico-chemical phenomena during contraction, and a comparison of plant cell contractility with other varieties of contraction, such as striped muscle contraction, contractile protozoa, etc.

Chapters V and VI are especially interesting. The former deals with various kinds of stimuli—electrical, mechanical, thermal, radiant, osmotic, and chemical. It also takes up the problems of intensity of stimulation as threshold phenomena, "all or none" relations, summation, tetany, fatigue, refractive stages, etc. The latter considers such changes in the protoplasm as variations of potential, pH and rH, and modifications in the concentration of crystalloids, colloidal state, and protoplasmic permeability as concomitants of contraction.

The book gives a well rounded consideration of its field, and is written in engaging style. A long list of citations offers opportunity for wide reading by those interested in research on such problems.—C. A. SHULL.

Die chromatographische Adsorptionsmethode. By L. ZECHMEISTER and L. v. CHOLNOKY. Vienna: Julius Springer, 1937. Pp. ix+231. Figs. 45. Rm. 14.4.

About 30 years ago TSWETT used the chromatographic method for separating mixtures of carotenoids. The method is now widely used, and this monograph, which gives a detailed account of it, should stimulate even wider application of the technique to research in the fields of plant and animal chemistry.

The monograph is divided into two parts, general and special. There are two chapters devoted to general considerations, such as the fields in which the chromatographic method is useful, the history of its use, the fundamental theoretical basis of chromatography, the relation between molecular constitution and chromatogram formation, and the details of the method as used with both colored and colorless compounds. The special part, presented in three chapters, takes up the application of the method to naturally occurring pigments, such as chlorophyll, porphyrins, bile pigments, carotenoids, naphthaquinones and anthraquinones, flavines, pterine, anthocyanins, and other unclassified pigments; to artificial pigments and dyes; and to colorless and weakly colored substances. Among the latter are found various aliphatic and aromatic compounds, benzol derivatives, polycyclic compounds, certain plant and animal poisons, alkaloids, enzymes, vitamins, hormones, tannins, and pharmaceutically useful drugs.

Several hundred citations and author and subject indexes make the work extremely useful and usable. It will commend itself particularly to students of the polyene carotenoids, and hormones.—C. A. SHULL.

Oklahoma Flora. By THOMAS R. STEMEN and W. STANLEY MYERS. Oklahoma City: Harlow Publishing Corporation, 1937. Pp. xxix+706. Figs. 494. $6.00.

Amateur botanists and beginning students of biology and botany in Oklahoma should be grateful to the two teachers in the Oklahoma City school system who have expanded their earlier work, The Spring Flora of Oklahoma, into the present volume. It seems to be intended primarily for the use of students. Over 1600 species are keyed and described, of which almost 500 are illustrated with original and borrowed line drawings. Tables listing edible, poisonous, and drug plants, those that should be protected, those causing hay fever, and those useful in fish culture and for transplanting are included, to appeal to the general public.

Professional botanists will regret that, in a work of such a pretentious nature, no attempt has been made to treat any grasses, sedges, or rushes, even though other species with only one known station in the state are included. Unfortunately the authors have been inconsistent in this latter respect, so that even though the work is said to be complete, numerous species have been omitted for which herbarium specimens are easily available. It is to be hoped that in any revision the authors will use all important available collections as a basis.—C. E. OLMSTED.

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